

# **How Science Conducted in the Lab is Changing the Structure of Clinical Trials**

## **Molecular Profiling of Thoracic Malignancies**

**Giuseppe Giaccone, MD PhD**

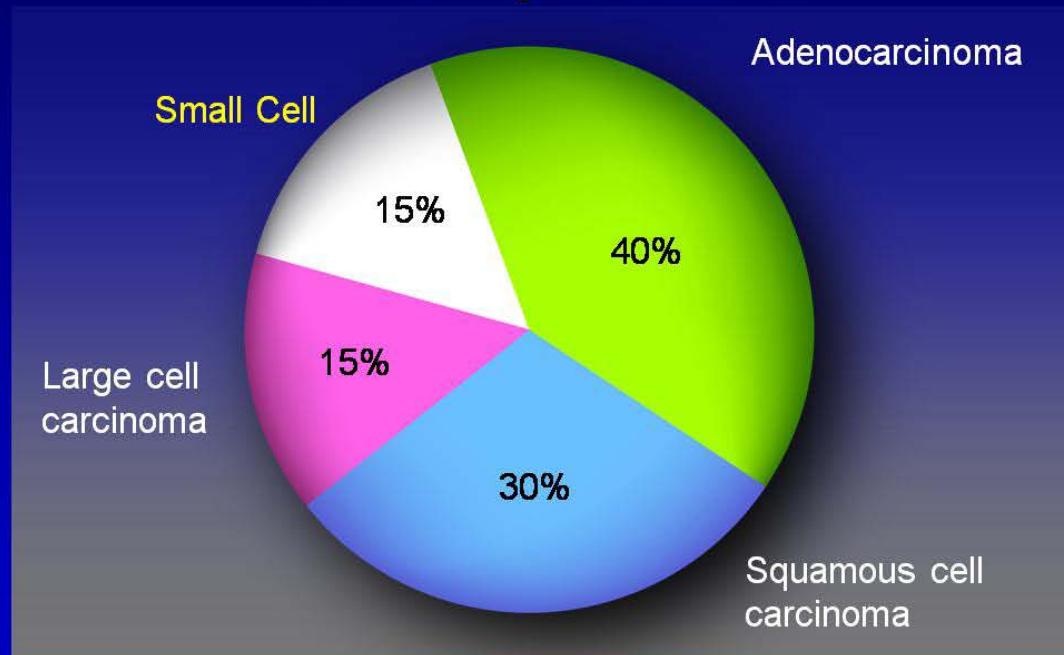
**Medical Oncology Branch  
National Cancer Institute  
Bethesda, MD**

**DCLG, Washington DC  
September 21, 2011**

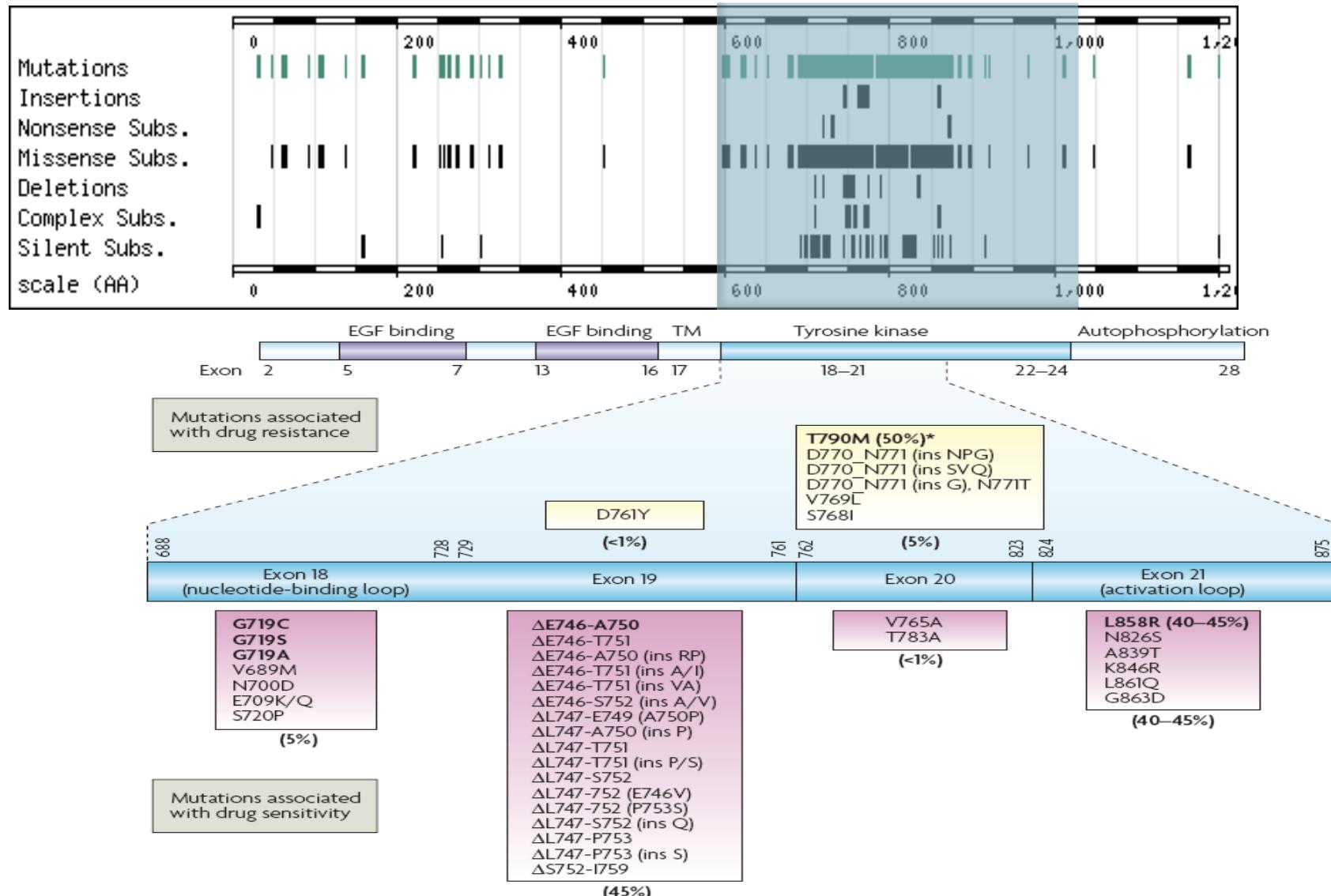


# The Changing Face of Lung Cancer

## Incidence of Histologic Subtypes in the US Population<sup>1</sup>



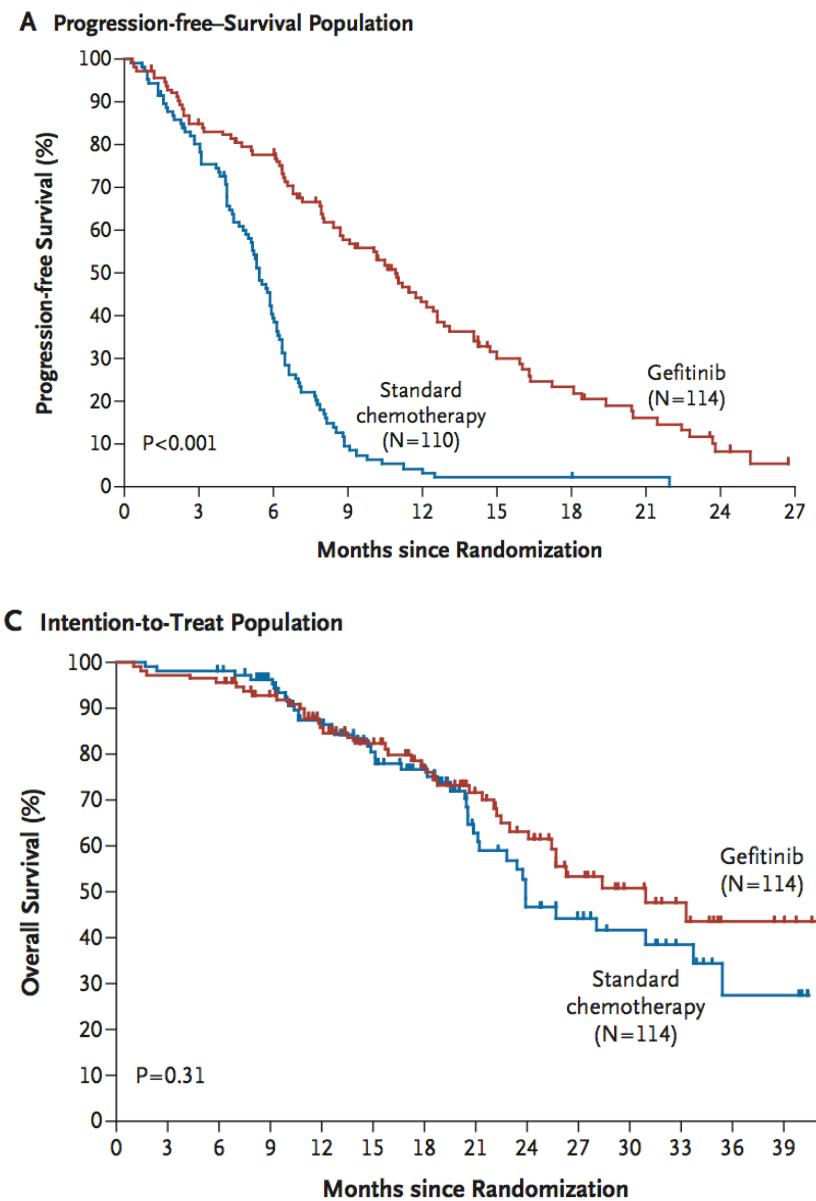
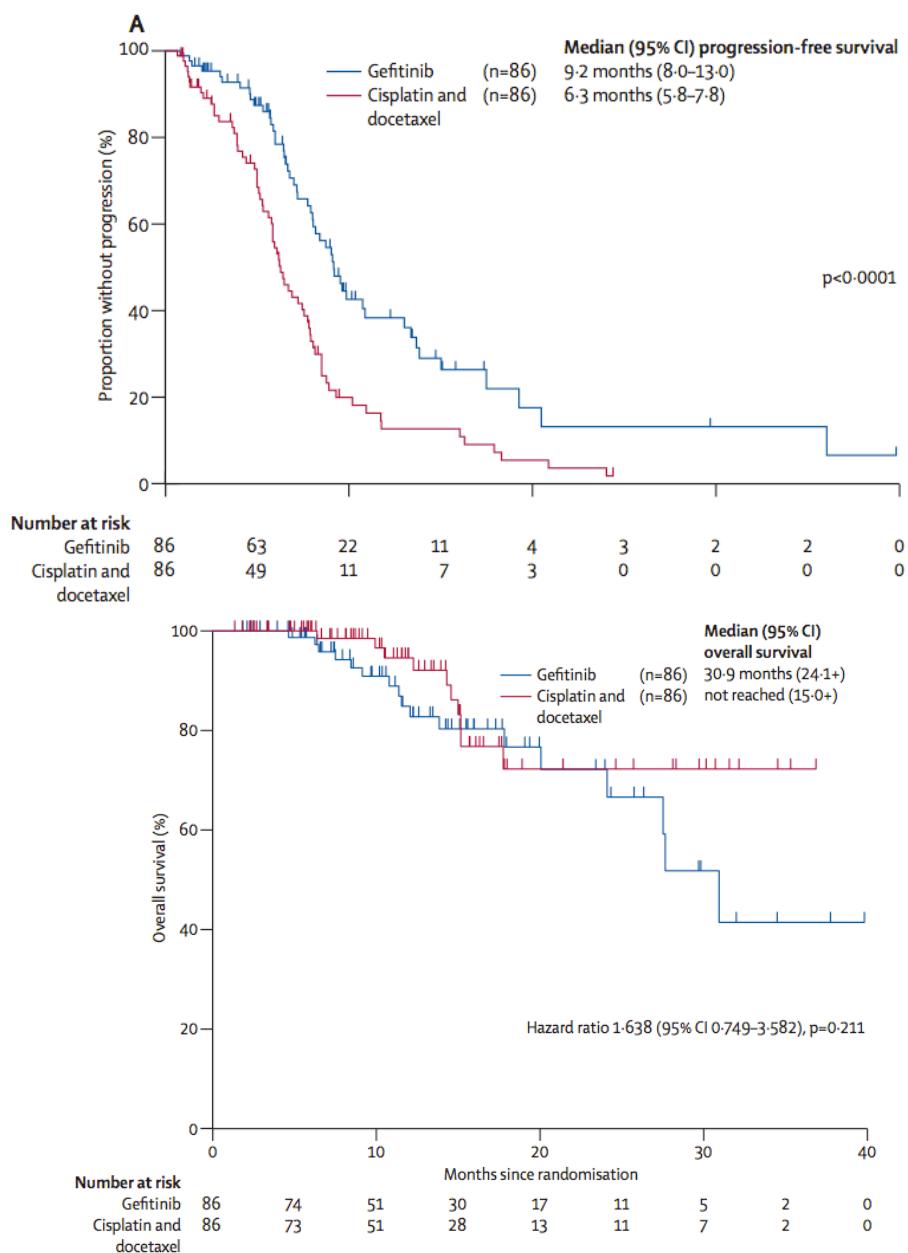
# EGFR mutations



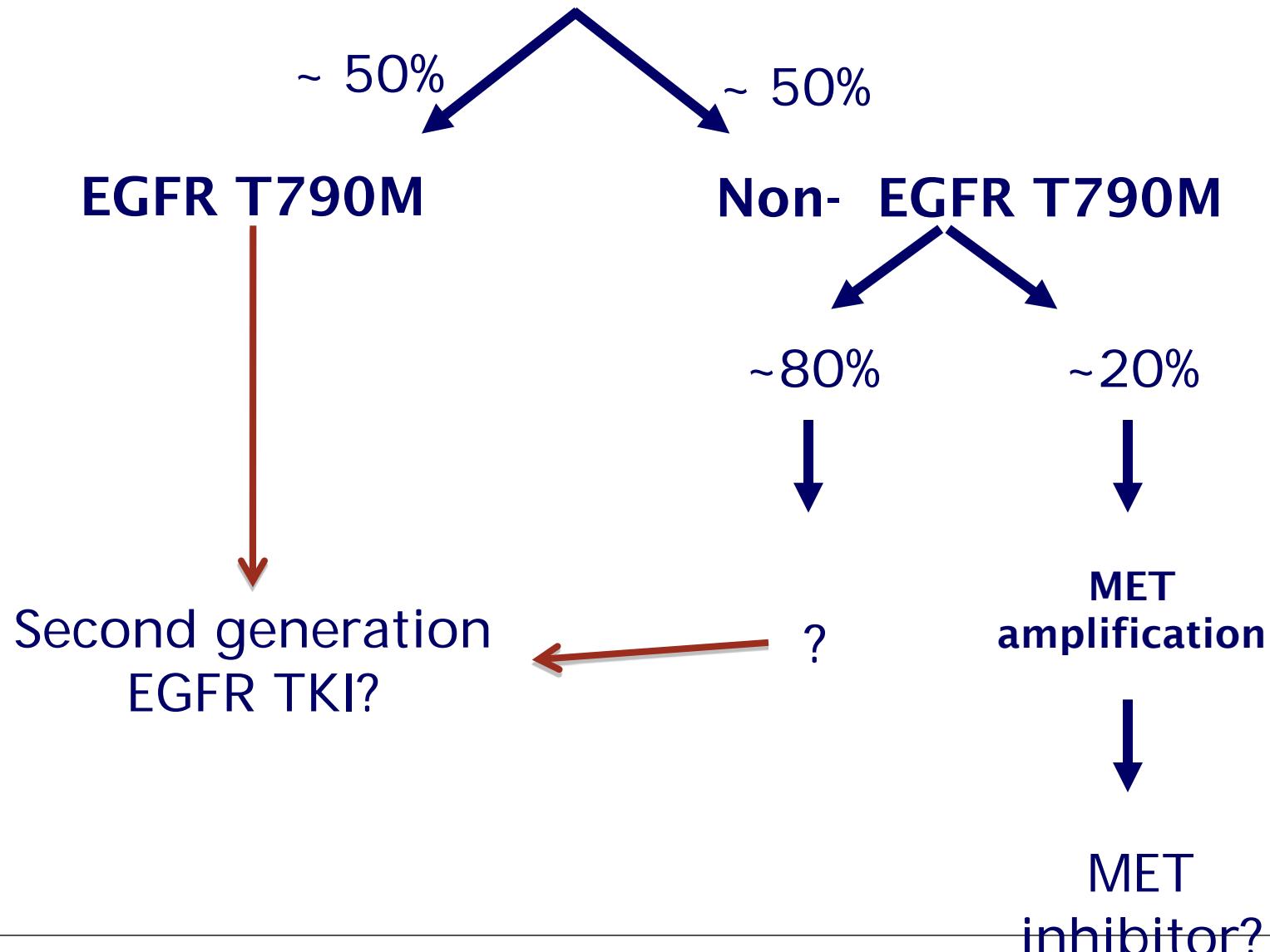
# Prospective clinical trials comparing EGFR-TKIs to chemotherapy in first-line in advanced NSCLC with EGFR mutations

Author	Treatments	# patients	Response rate	PFS (m)	OS (m)
Mitsudomi (Lancet Oncol 2010)	Gefitinib DDP/DXL	86 86	62.1 23.2 (p<.001)	9.2 6.3 (p<.001)	30.9 NR (p=NS)
Maemondo (NEJM 2010)	Gefitinib CRB/PXL	114 110	73.7 30.7 (p<.001)	10.8 5.4 (p<.001)	30.5 23.6 (p=NS)
Zhou (Lancet Oncol 2011)	Erlotinib CRB/GEM	82 72	83 36 (p<.0001)	13.1 4.6 (p<.0001)	NA NA
Rosell (ASCO 2011)	Erlotinib CRB/GEM DDP/DXL	77 76	54.5 10.5 (p<.0001)	9.4 5.2 (p<.0001)	22.9 18.8 (p=NS)
NR=not reached; NA=not available; NS=not significant					

# EGFR mutant selected



# Mechanisms of Acquired Resistance to EGFR TKIs



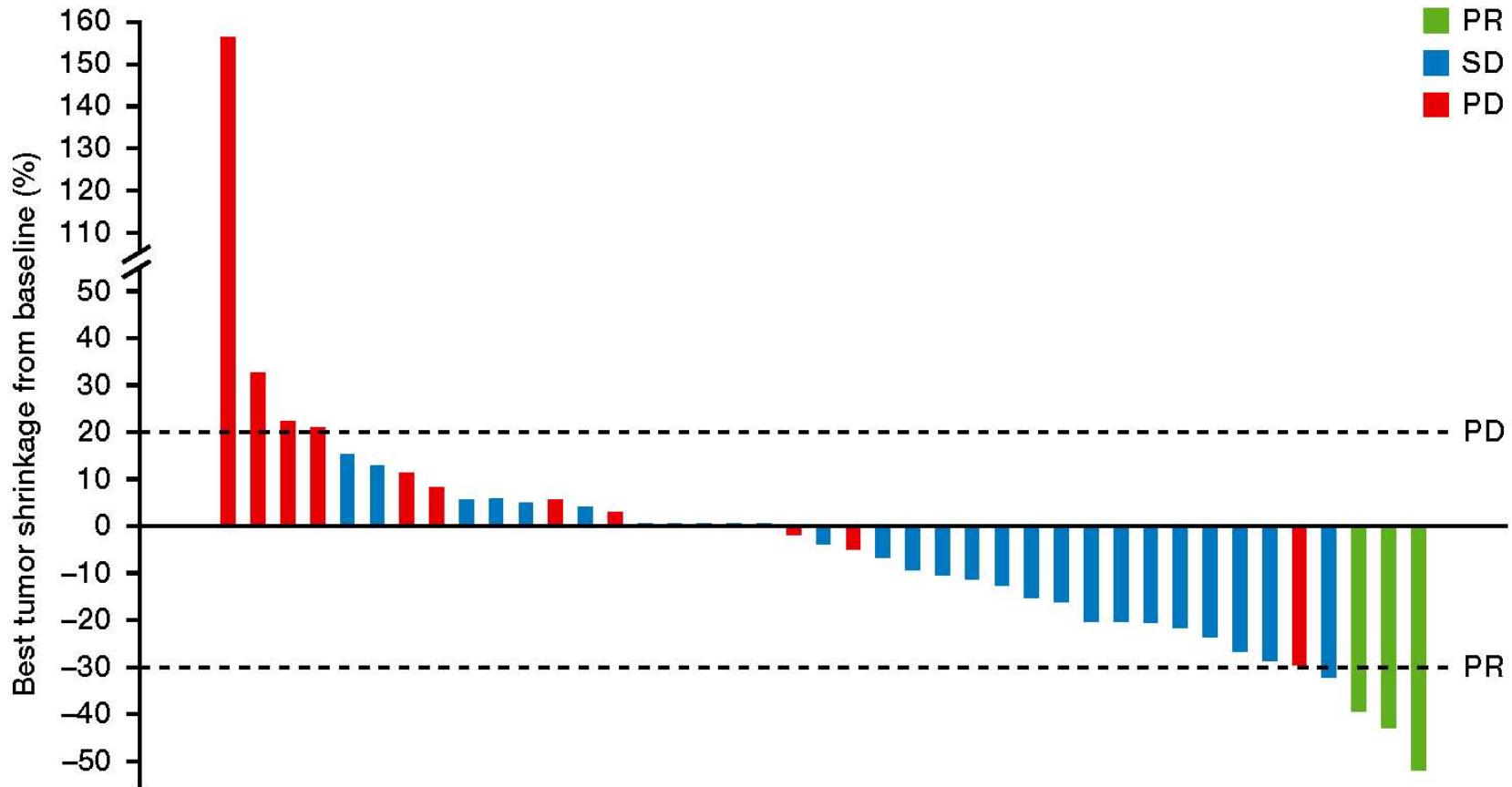
# Dacomitinib (PF-00299804)

## *in vitro* Activity against EGFR, HER2 and KRASmut NSCLC Cell Lines

Cell Line	EGFR mutation	ERBB2 mutation	K-ras mutation	Gefitinib IC <sub>50</sub>	PF00299804 IC <sub>50</sub>
A549	WT	WT	G12S	>10 μM	>10 μM
H441	WT	WT	G12V	>10 μM	4 μM
Calu-3	WT	WT HER2+++	WT	1.4 μM	0.063 μM
H1819	WT	WT HER2+++	WT	0.42 μM	0.029 μM
H1781	WT	Ins G776V,C	WT	>10 μM	0.275 μM
HCC 827	Del E746_A750	WT	WT	0.008 μM	0.002 μM
HCC 4006	Del L747_E749	WT	WT	0.050 μM	0.004 μM
PC-9	Del E746_A750	WT	WT	0.023 μM	0.002 μM
H3255	L858R	WT	WT	0.075 μM	0.007 μM
H3255 GR	L858R/T790M	WT	WT	>10 μM	0.119 μM
H1975	L858R/T790M	WT	WT	>10 μM	0.44 μM

- MTS 72-Hour Proliferation Assay; 6-12 wells per assay; all experiments repeated at least 3X

## Dacomitinib - response



PD = progressive disease; PR = partial response; SD = stable disease.

# Case Presentation

- 52 y/o AA male
  - Never smoker
  - Diagnosed with NSCLC adenocarcinoma
  - Stage IV, multiple lesions in LLL and RUL
- No Past Medical History
- No Family History
- Treatment:
  - carboplatin/paclitaxel/bevacizumab
    - 8 cycles, initial Partial Response (PR) then Progressive Disease (PD)
  - erlotinib 150 mg PO daily, (6 weeks) PD
  - Referred for evaluation at NCI

# Treatment Course

- Screened for Dacomitinib trial:
  - KRAS wild type (wt)
  - [EGFR wt]
- Enrolled on trial Dacomitinib for patients who have failed EGFR TKIs
- Started on Dacomitinib
  - 45 mg PO daily

## Pretreatment and after 4 cycles of Dacomitinib

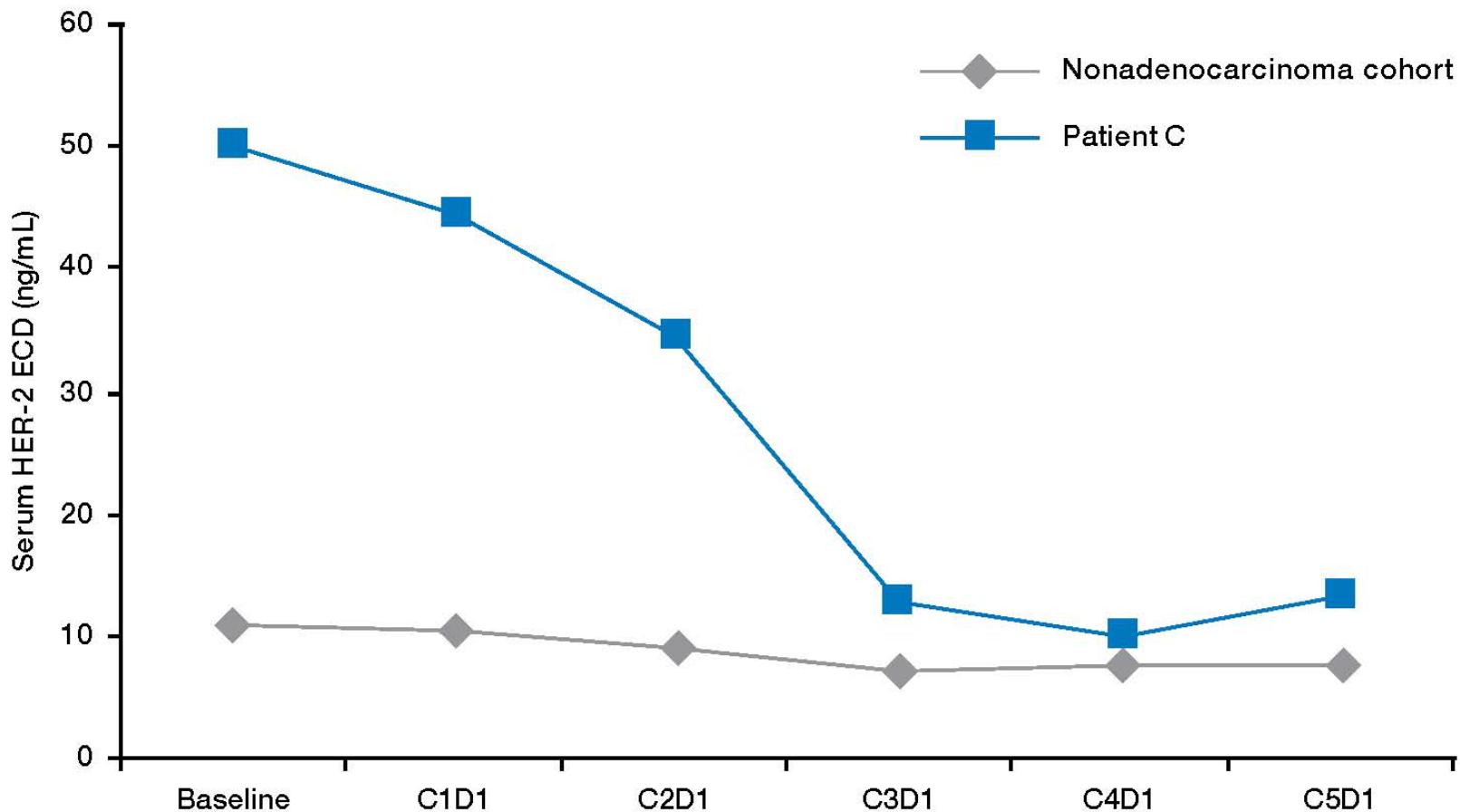


October 21, 2008



January 16, 2009

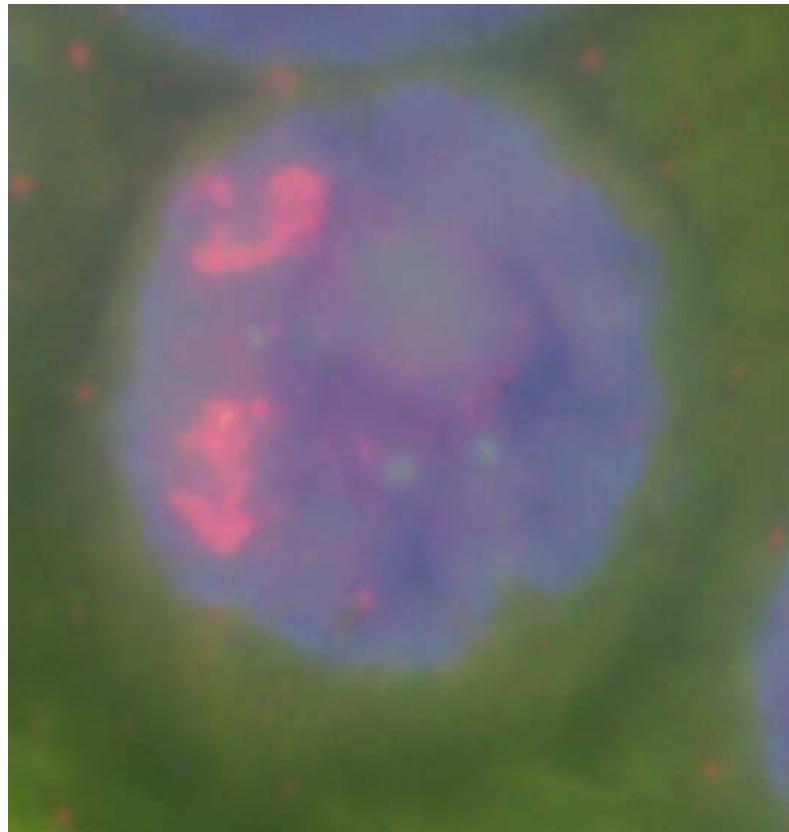
# Serum HER-2 levels at baseline and through 4 cycles of Dacomitinib therapy



C = cycle; D = day; ECD = extracellular domain; EGFR = epidermal growth factor receptor; WT = wild type.

# Stain Tumor for Her2/Neu

## HER2/Neu FISH



## PATHOLOGY

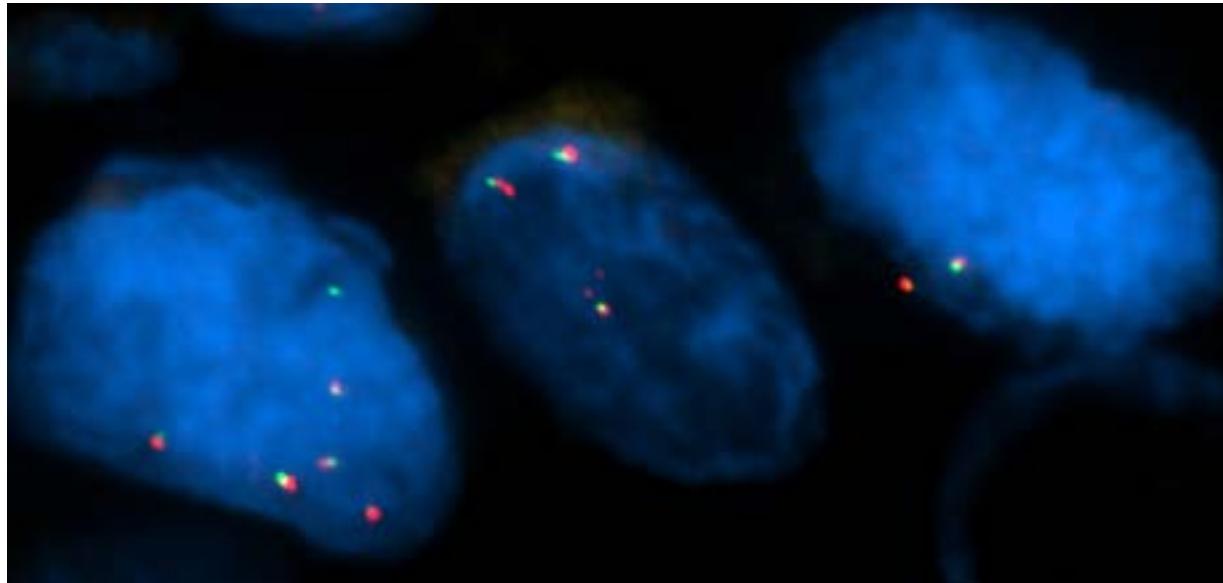
- IHC 2+
- Her2/Neu Amplified
  - Cells counted 20
  - HER2 = 10.3
  - CEP17 = 2.4
  - HER2/CEP17 Ratio: 4.3

# Follow-up treatment

- Trastuzumab – tumor progression
- Trastuzumab/vinorelbine – partial response > 1 year
- Progression
- Lapatinib – partial response on lung lesion, but progression in neck lymphnodes

# Old Lung Tumor

## SI-10-0625



ALK-negative

14/300 cells (<5%) showing split ALK red/green signals  
GAIN (extra copies) of wt ALK: 3-5 copies/cell

# Recent Left cervical LN (biopsy)

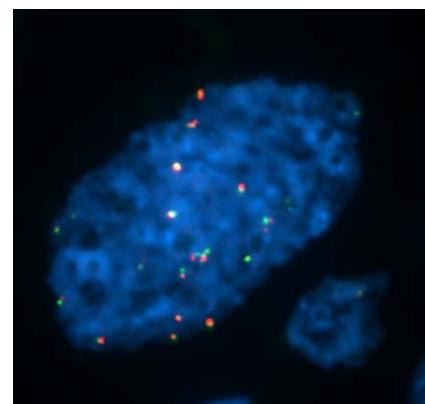
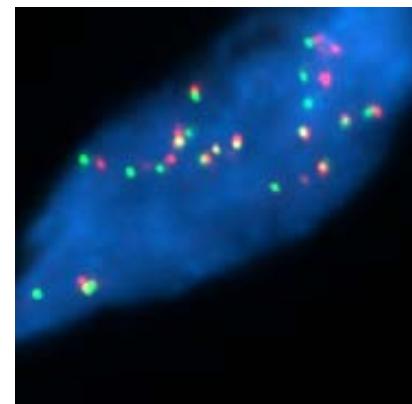
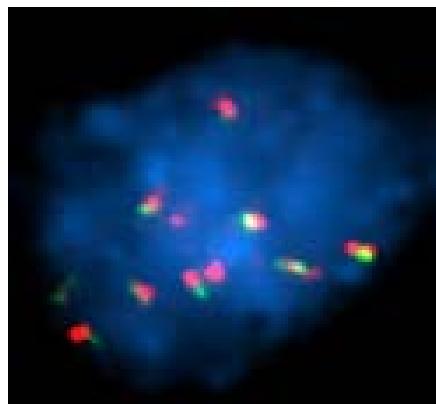
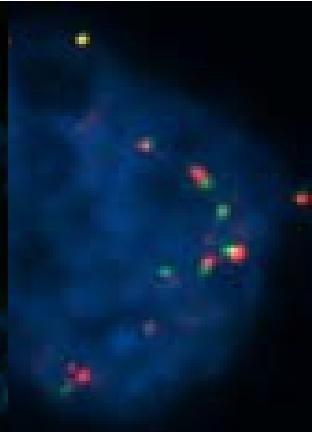
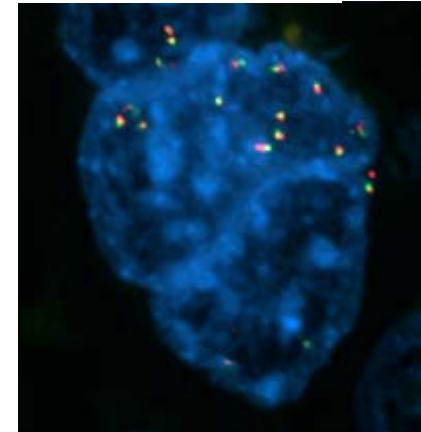
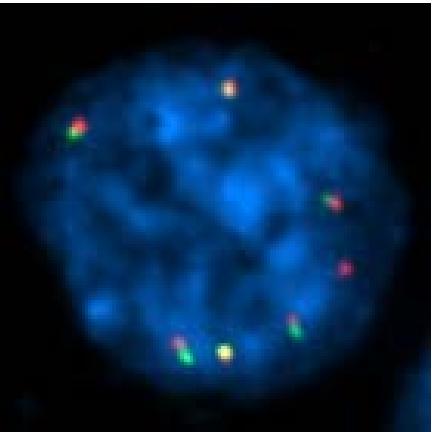
NIH 2011

Heterogeneous population of tumor cells

- ALK rearranged in 62/300 cells scored (**20%**)
- High level of wt ALK >4 (4-20 copies) amplification detected in 40% of tumor cells
- HER2 amplification – average **number of HER2 copies-9.2/CEP17-2.6/RATIO 3.5**

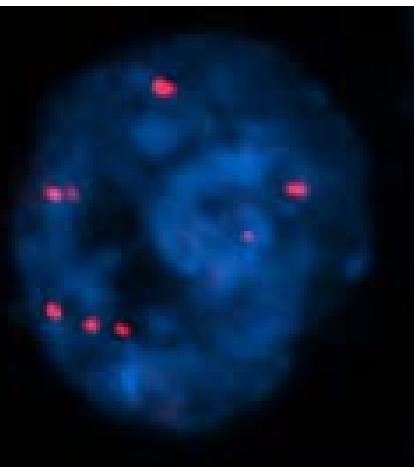
# SI-11-514 LN R NECK

## ALK BA Abbott

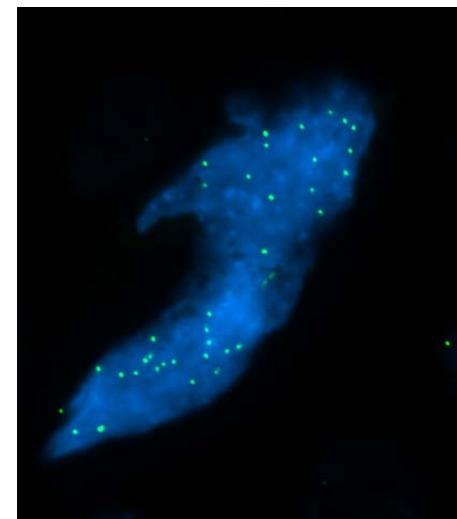
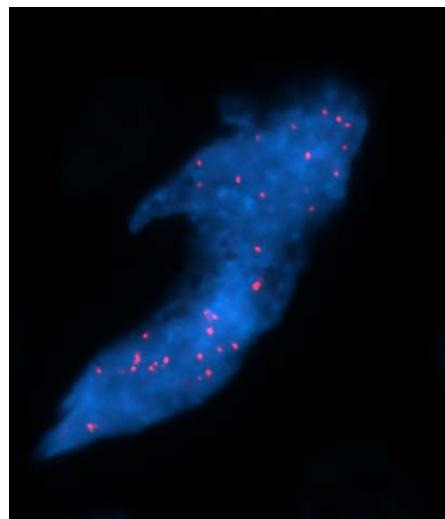
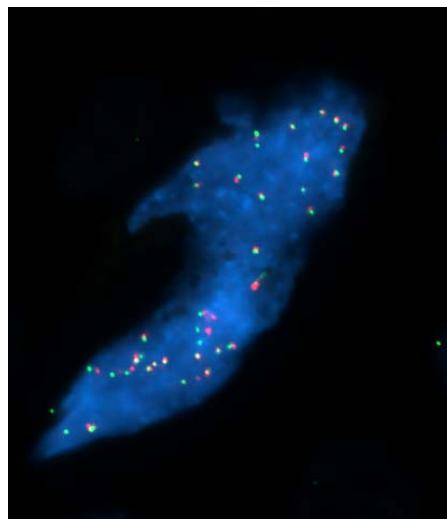
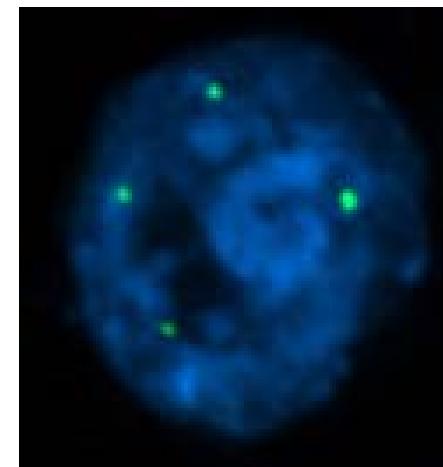


# ALK BA Abbott

SI-11-514 LN R NECK



Rearranged  
(19%)

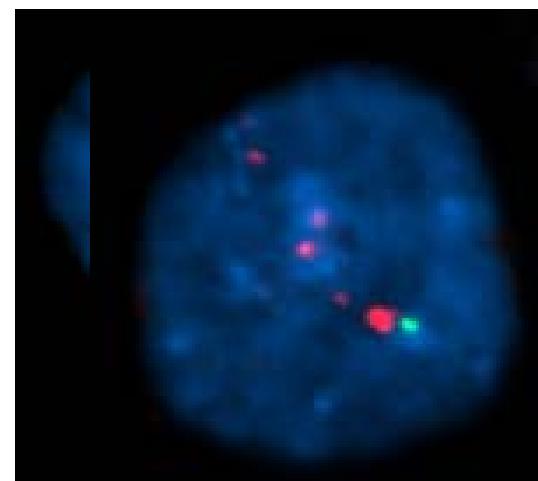
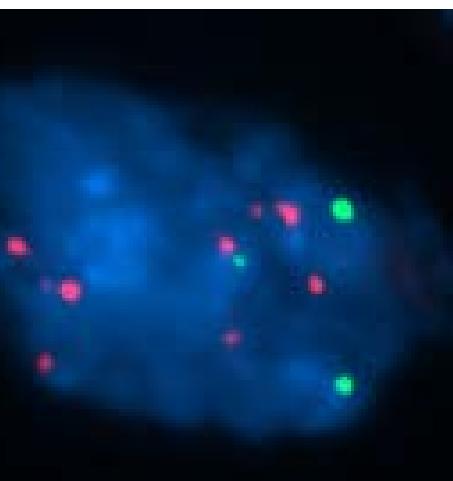
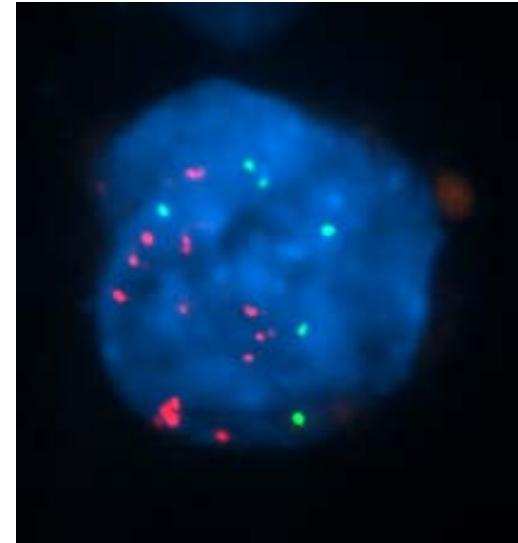
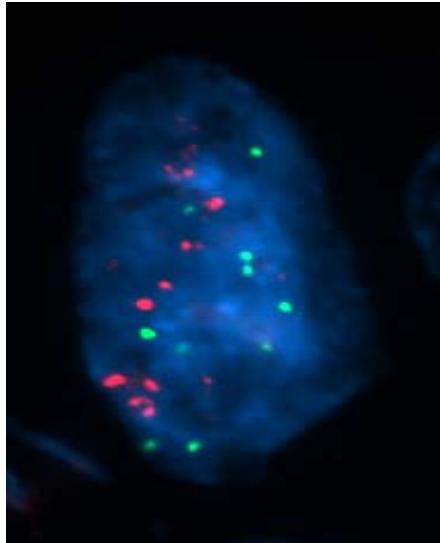
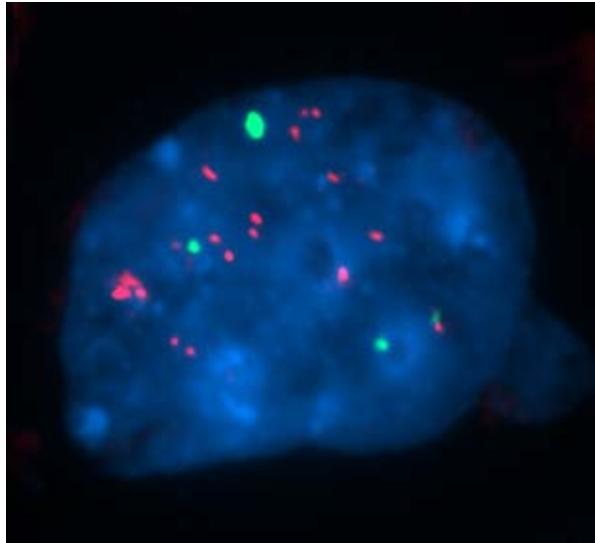


Amplified

SI-11-514

# HER2 amplification

(9.2 HER2 copies/cell average)

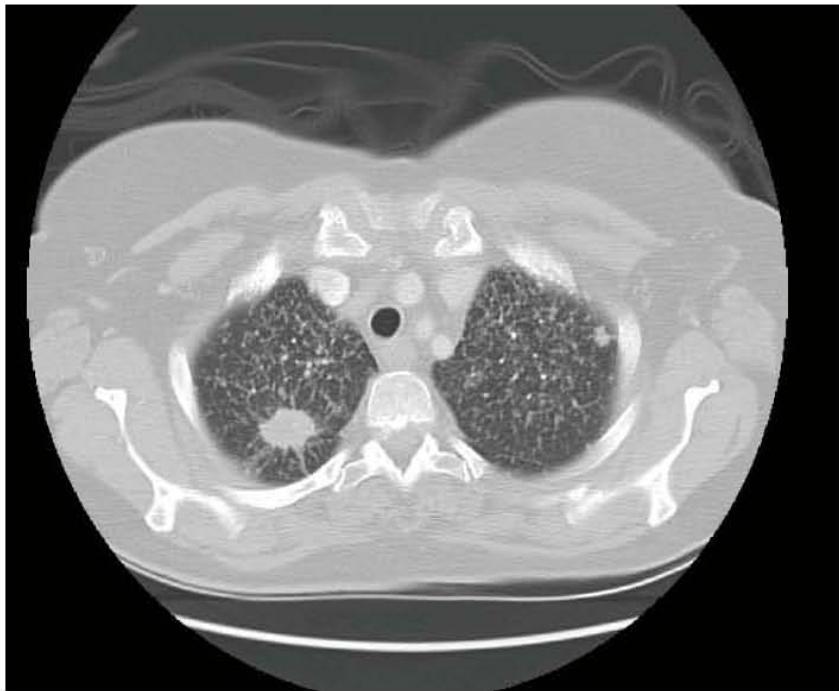


SI-11-514 LN R NECK

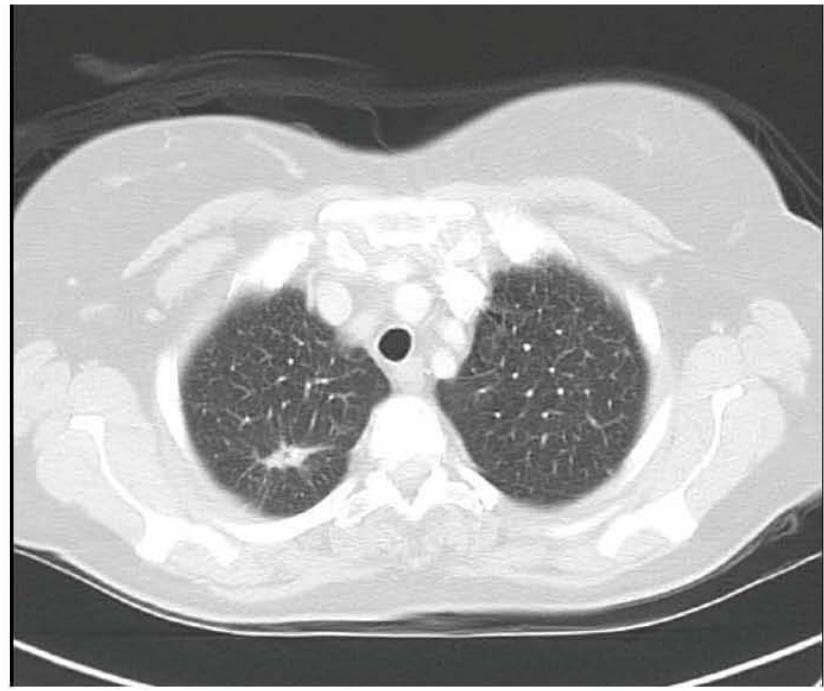
# Follow-up treatment

- Trastuzumab – tumor progression
- Trastuzumab/vinorelbine – partial response > 1 year
- Progression
- Lapatinib – partial response on lung lesion, but progression in neck lymphnodes
- Lymphnodes positive for ALK translocation, amplification and Her2 amplification
- Progression on crizotinib
- Response on lapatinib and capecitabine

# Durable SD (3 years +; 50 cycles +)



April 9, 2008



March 30, 2009

**adenocarcinoma, female, never-smoker, bone mets; 1 chemotherapy regimen,  
6.5 m erlotinib; 10 m alpha-galactosyl transferase vaccine**

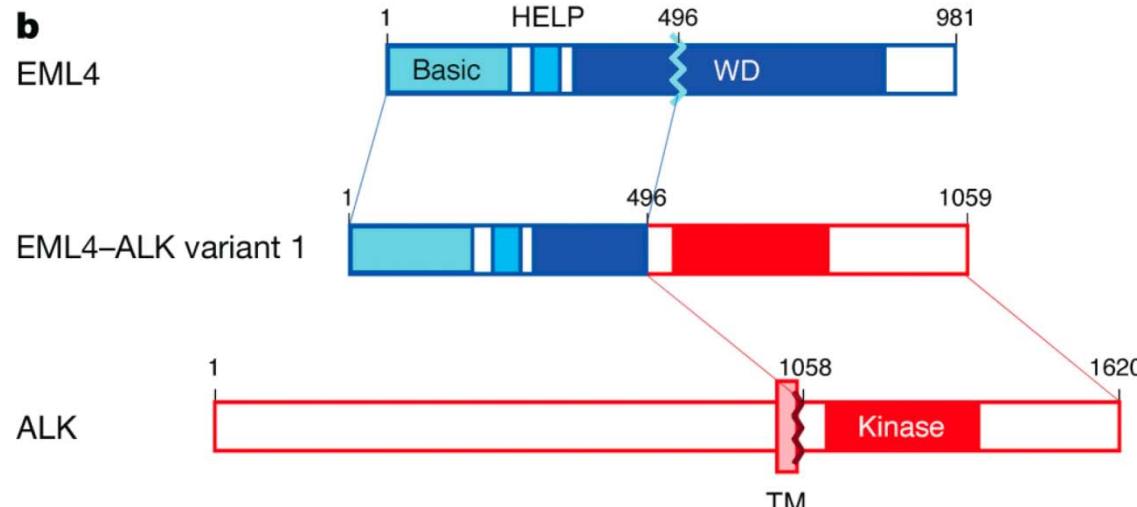
# *EML4-ALK* Translocations in NSCLC

Vol 448 | 2 August 2007 | doi:10.1038/nature05945

nature

## Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer

Manabu Soda<sup>1,2</sup>, Young Lim Choi<sup>1</sup>, Munehiro Enomoto<sup>1,2</sup>, Shuji Takada<sup>1</sup>, Yoshihiro Yamashita<sup>1</sup>, Shunpei Ishikawa<sup>5</sup>, Shin-ichiro Fujiwara<sup>1</sup>, Hideki Watanabe<sup>1</sup>, Kentaro Kurashina<sup>1</sup>, Hisashi Hatanaka<sup>1</sup>, Masashi Bando<sup>2</sup>, Shoji Ohno<sup>2</sup>, Yuichi Ishikawa<sup>6</sup>, Hiroyuki Aburatani<sup>5,7</sup>, Toshiro Niki<sup>3</sup>, Yasunori Sohara<sup>4</sup>, Yukihiko Sugiyama<sup>2</sup> & Hiroyuki Mano<sup>1,7</sup>



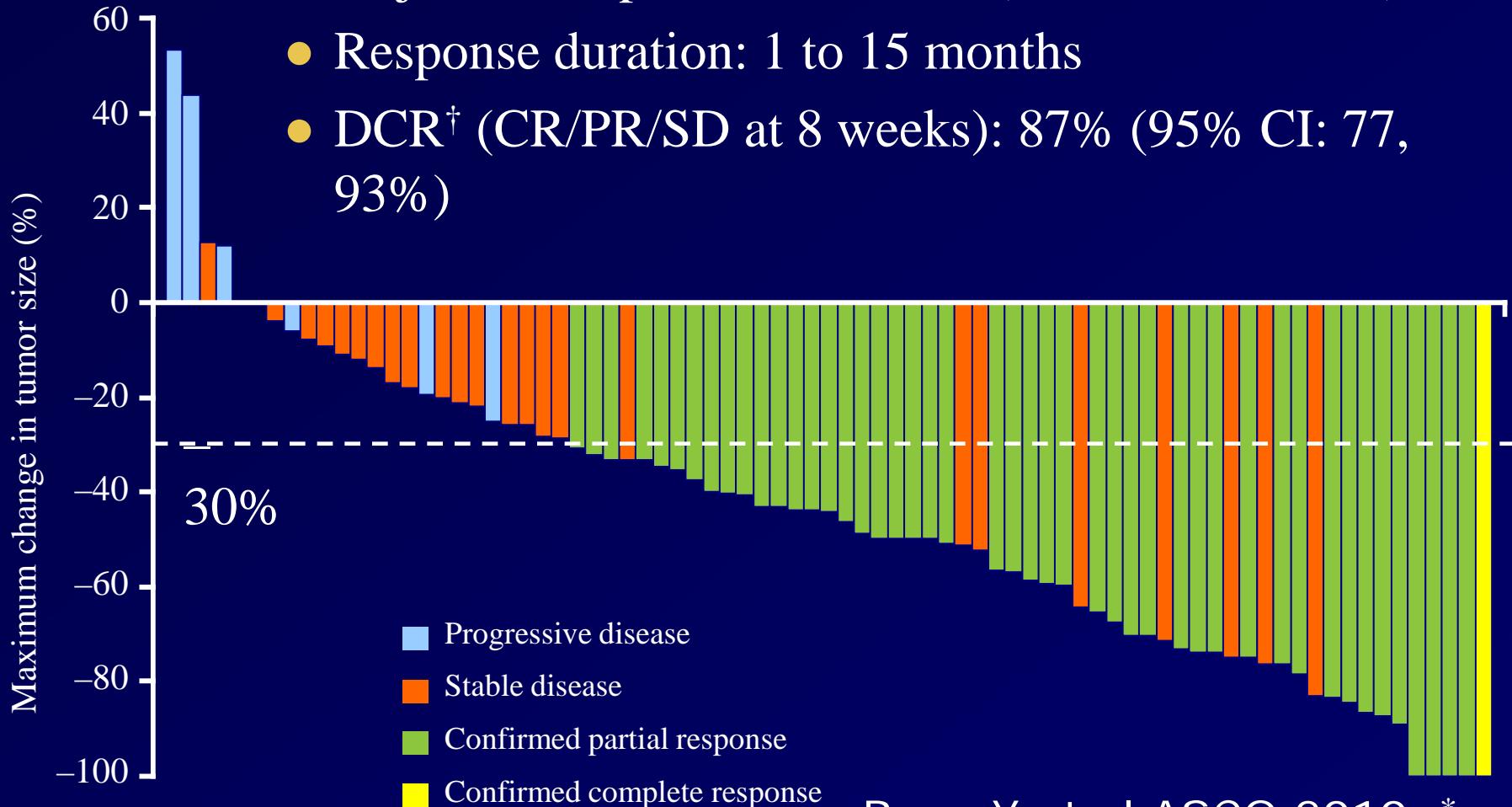
***EML4-ALK* frequency:**

**~4% (64/1709)**

**Primarily lung adenocarcinoma**

# Tumor Responses to Crizotinib for Patients with *ALK*-positive NSCLC

- Objective response rate : 57% (95% CI: 46, 68%)
- Response duration: 1 to 15 months
- DCR<sup>†</sup> (CR/PR/SD at 8 weeks): 87% (95% CI: 77, 93%)



Bang Y et al ASCO 2010 \*

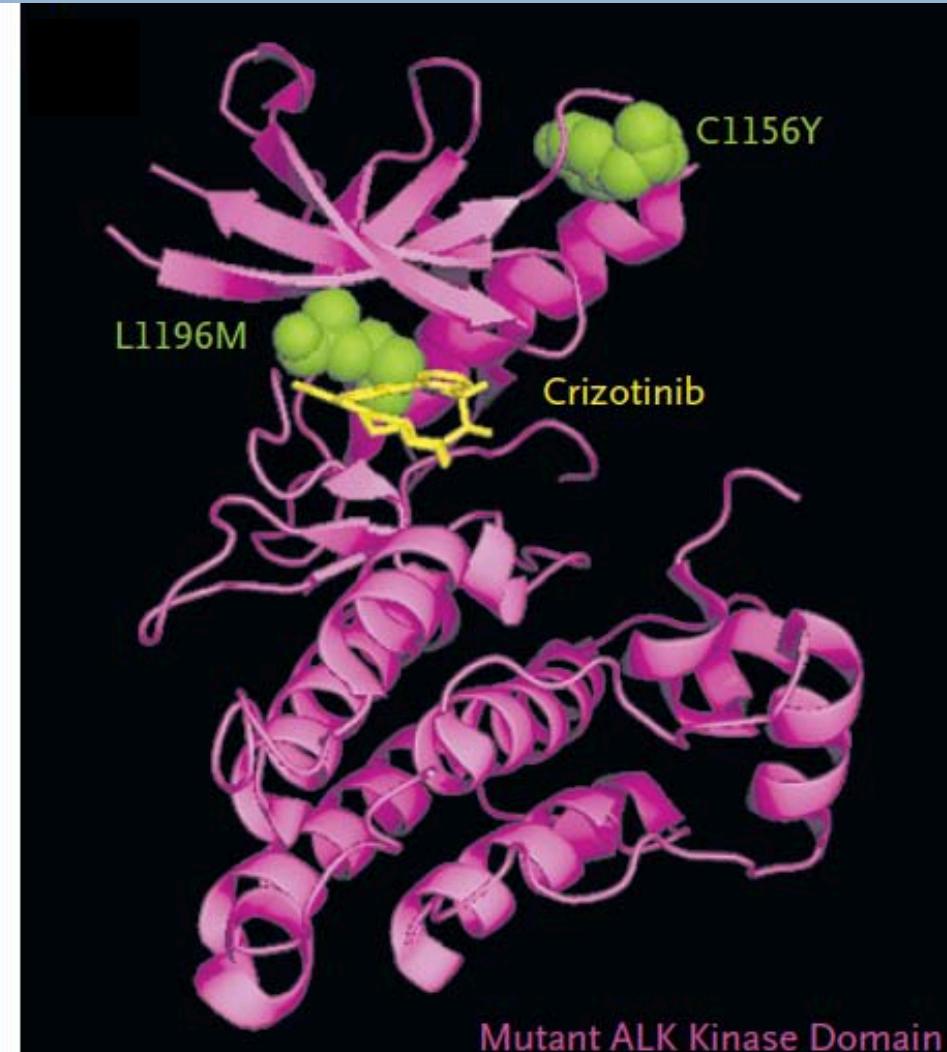
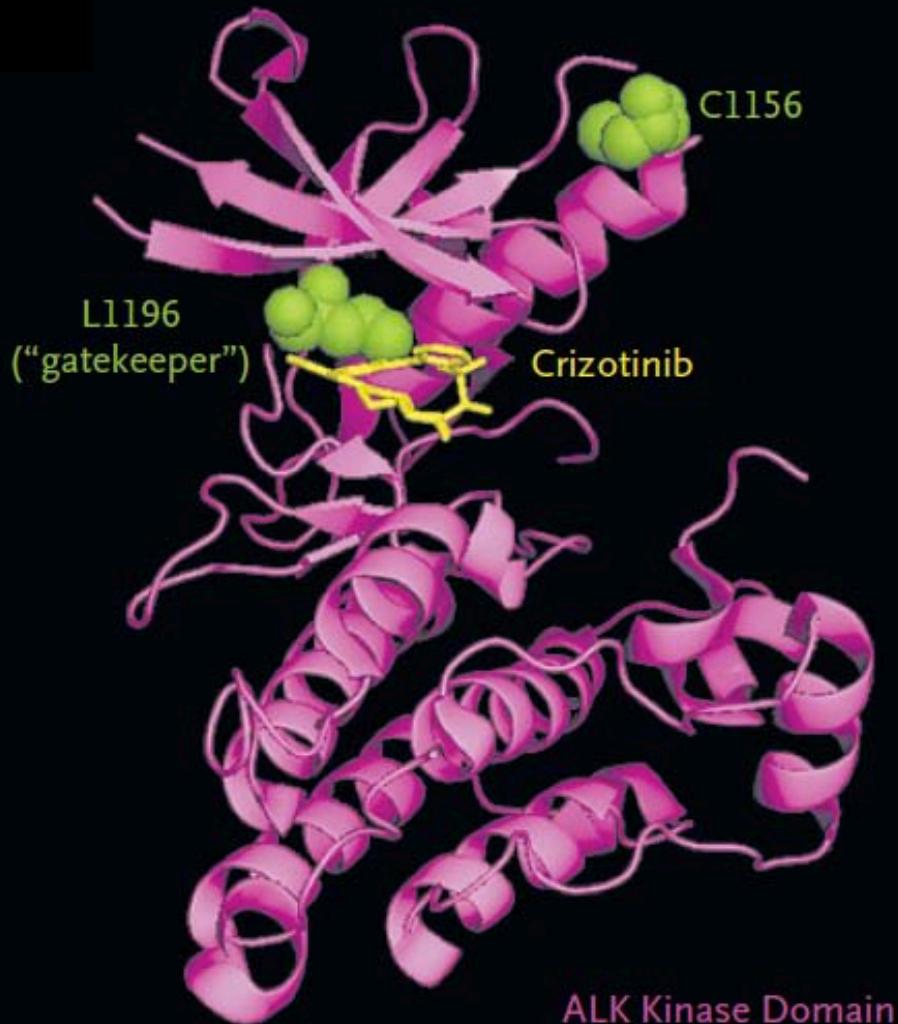
\*Partial response patients with 100% change have non-target disease present

# Main patient characteristics

- NSCLC screened = 1500
- NSCLC with Alk translocation = 82 + 2
- Male 52%
- White 56%, Asian 35%
- ≥ prior lines 41%
- Adenocarcinoma 96%
- Never-smoking 76%

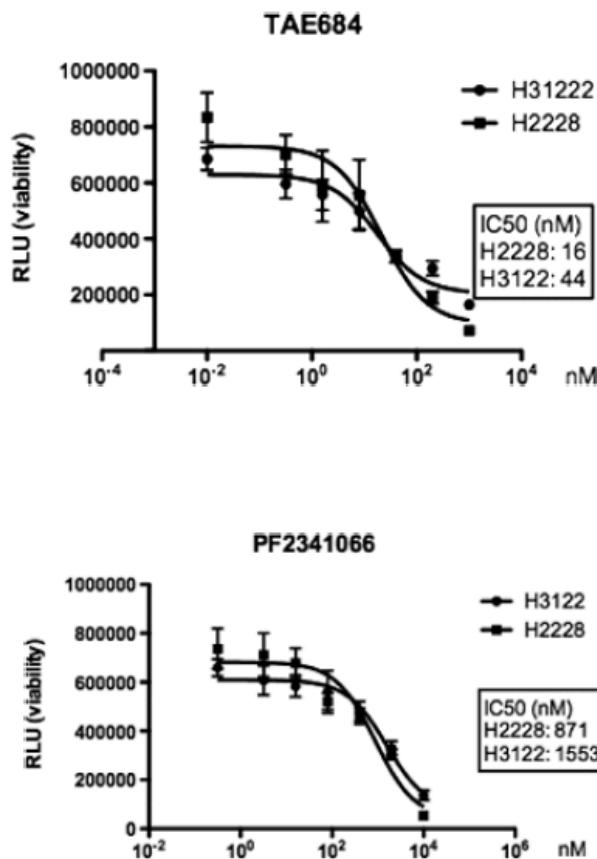
Kwak EL et al NEJM 363, 1693, 2010

# Predicted Crystal Structure of the Kinase Domain of ALK and Resistant Mutants

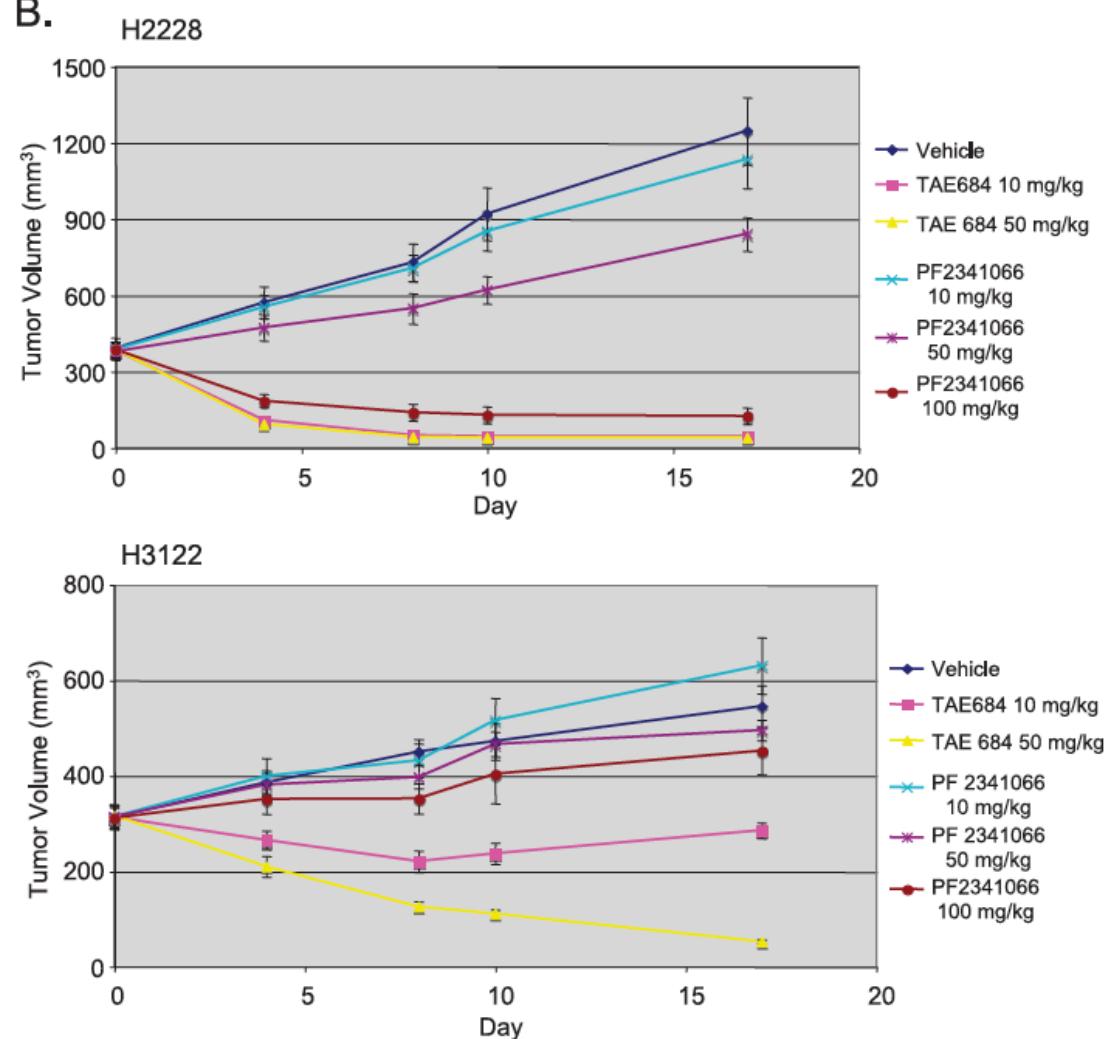


# TAE684 is more potent than Crizotinib

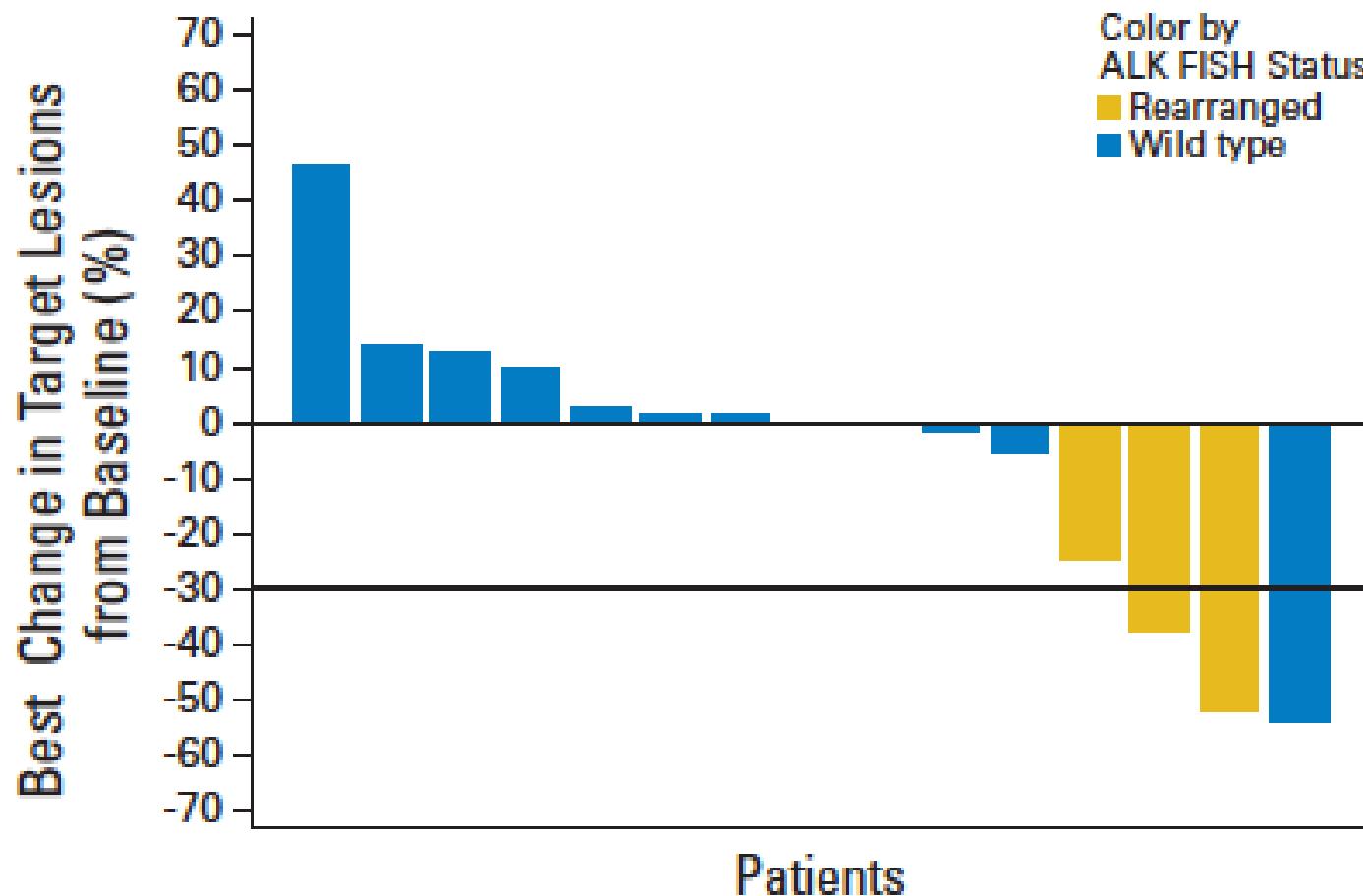
A.



B.

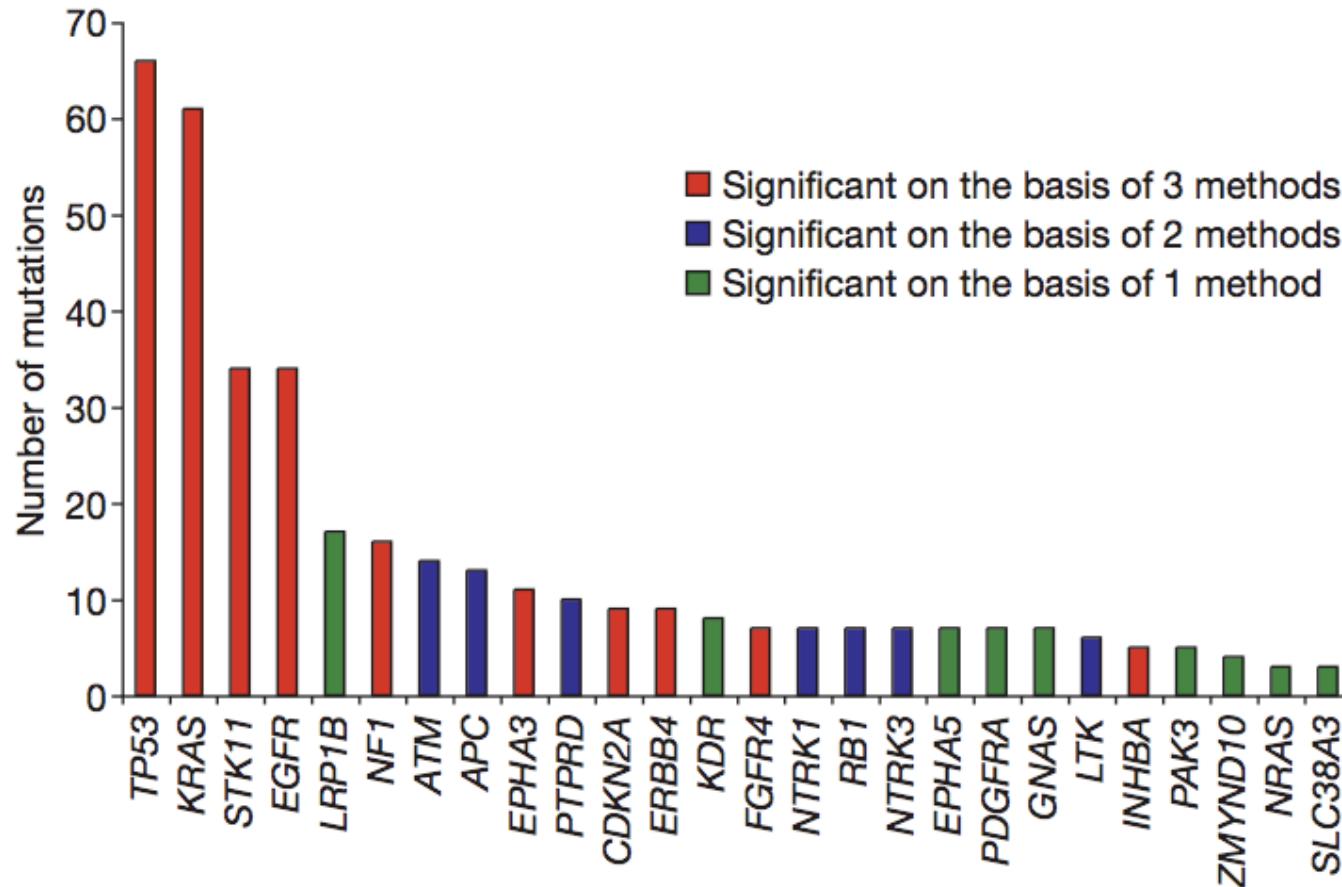


# Responses to IPI-504 (HSP-90 inhibitor) in ALK + NSCLC



Sequist LV et al. 28, 4953, 2010

# Significantly mutated genes in adenocarcinoma of the lung



Ding et al. Nature 455, 1069, 2008

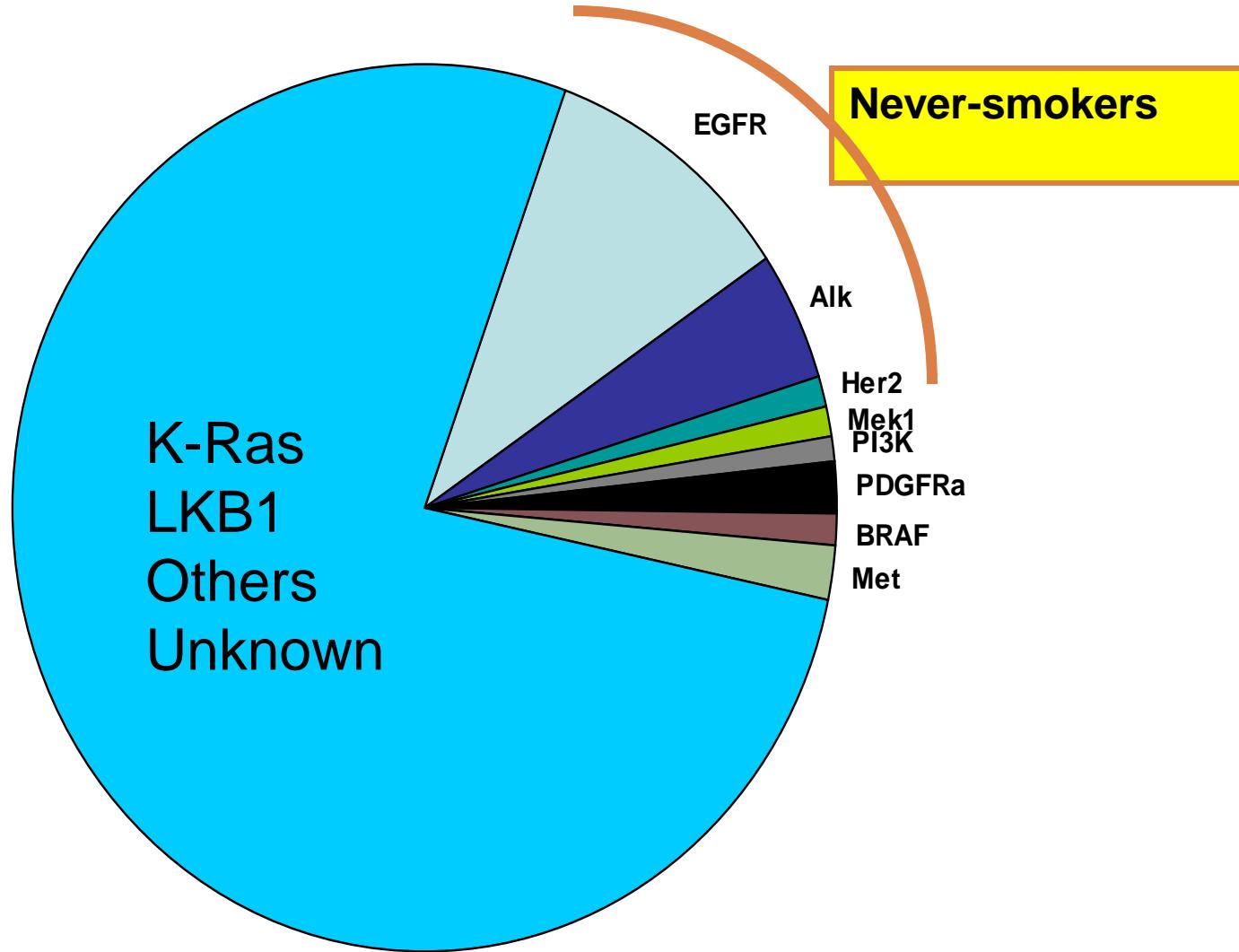
# “Oncogene addiction” may explain clinical responses to many kinase-targeted therapeutics

<u>Tumor type</u>	<u>Mutated/amplified kinase</u>	<u>Drug</u>
NSCLC	EGFR	gefitinib/erlotinib
NSCLC	Alk	crizotinib
CML	BCR-ABL	imatinib/dasatanib
GIST	c-Kit/PDGFR	imatinib/sunitinib
CMM	PDGFR-b	imatinib
Breast cancer	Her2	trastuzumab/lapatanib
Gastric cancer	c-MET	PHA-665752*
Gastric cancer	FGFR2	AZD2171*
Melanoma	B-Raf	PLX4032
Melanoma	Her4	lapatinib *
Basal cell carcinoma	PTCH1/SMO	GDC-0449

\* Pre-clinical data only

“Addiction” to mutationally activated kinases can be faithfully modeled in cell culture.

# “Drugging” the genome in NSCLC



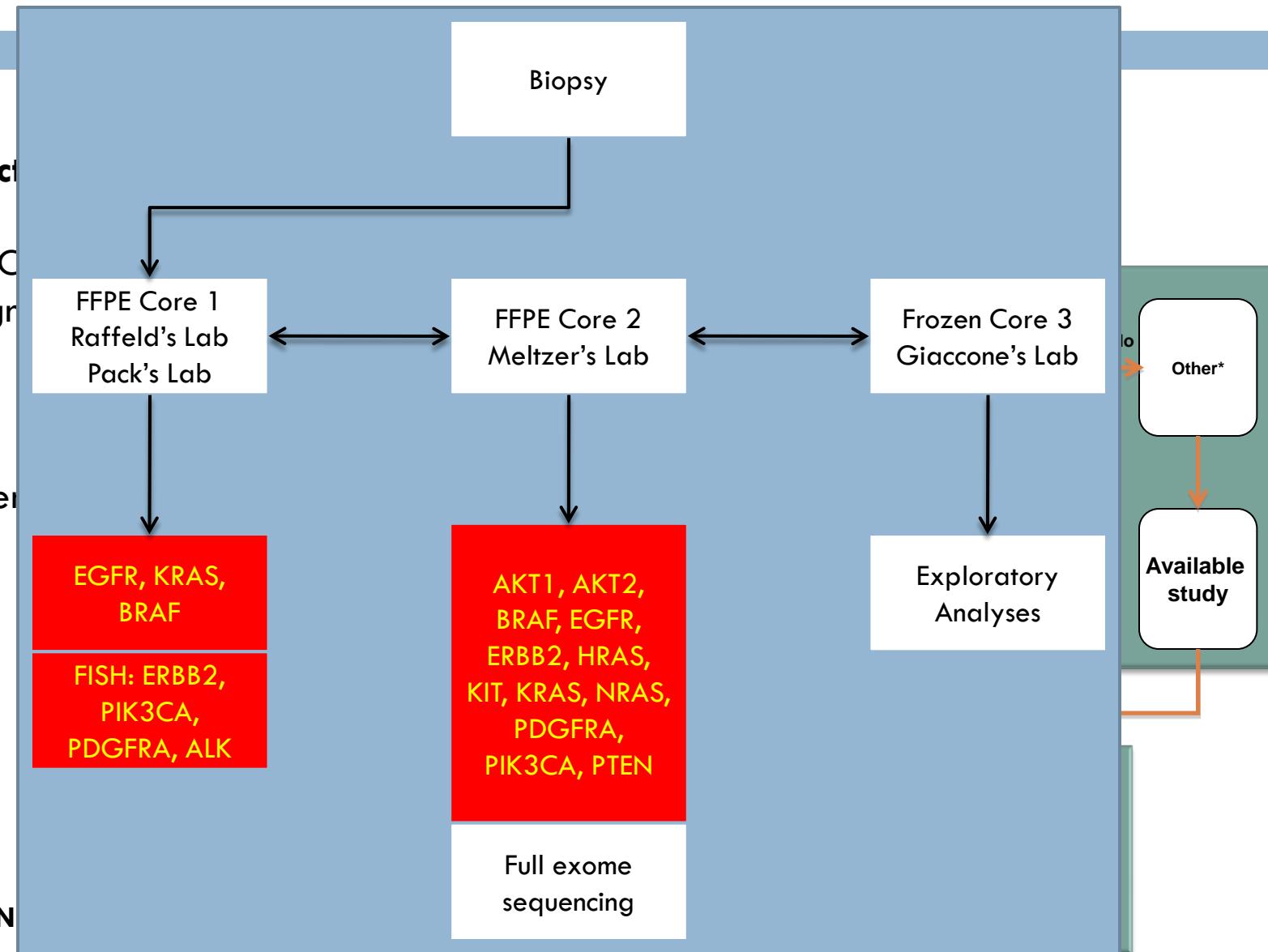
# Molecular Profiling of Thoracic Malignancies

- **Eligibility**
- Advanced, non-resectable NSCLC, SCLC and thymic malignancies.
- Tissue Requirements:
  - Biopsiable disease
  - Adequate archival material
  - Previously performed molecular profiling
- ECOG ≤ 2
- Normal organ and marrow function

# Molecularly Targeted Treatment of Advanced Thoracic Malignancies

Patient selection  
Molecular +  
NSCLC, SCLC  
thymic malignancies

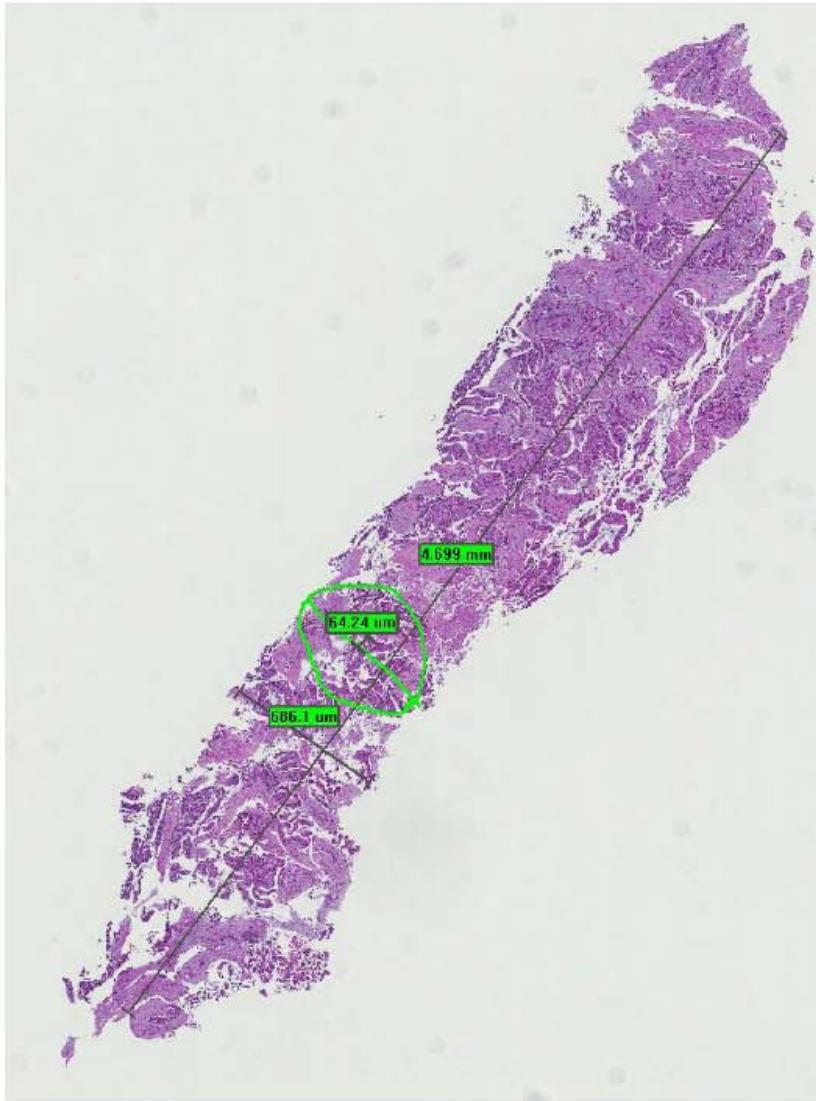
Treatment:  
Targeted therapy



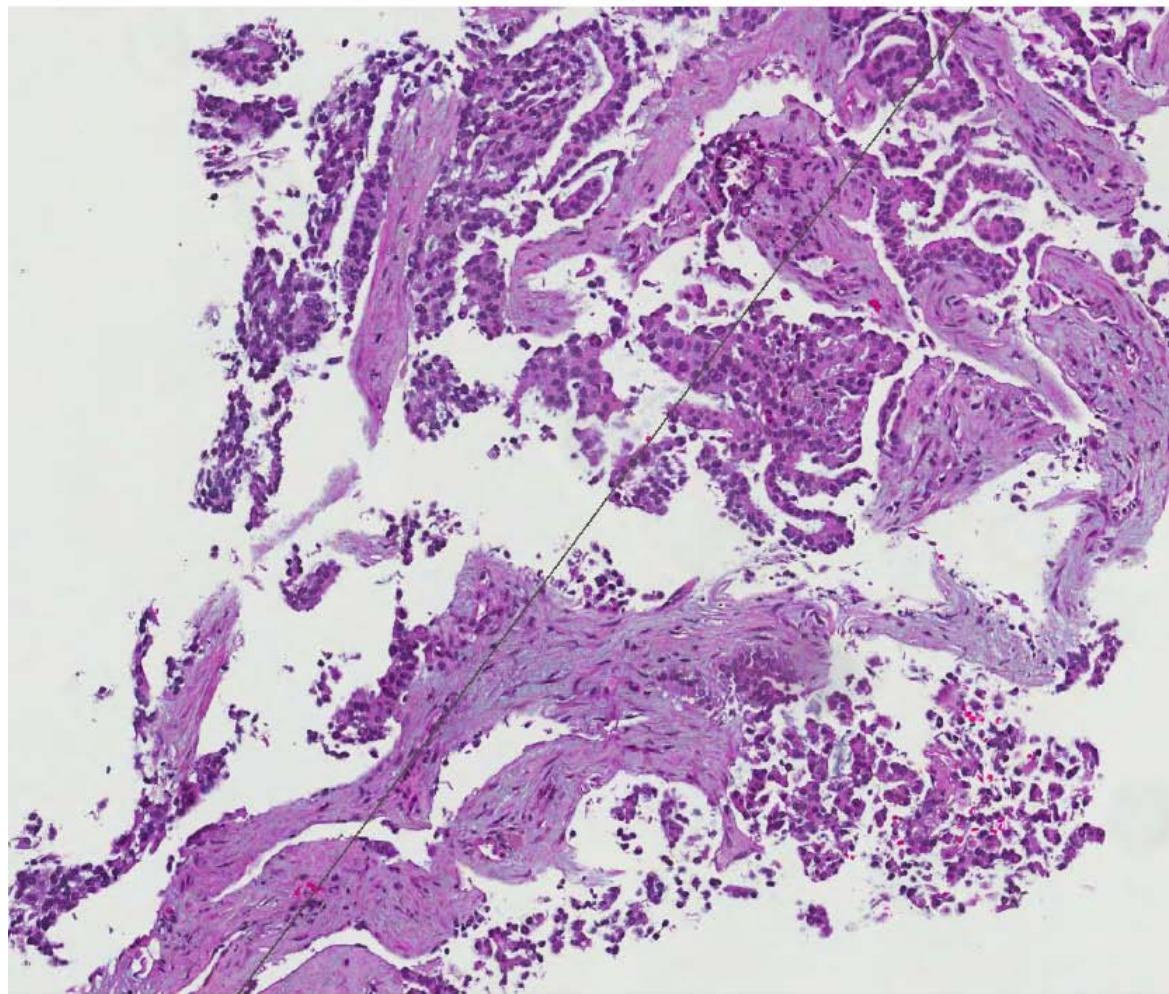
# Study Size

- 600 Patients
- 5 years
- Each patient may undergo repeat biopsies
- Close collaboration with Interventional Radiology and surgery imperative for the study to succeed

# CMPC11\_2969\_WHOLE MOUNT

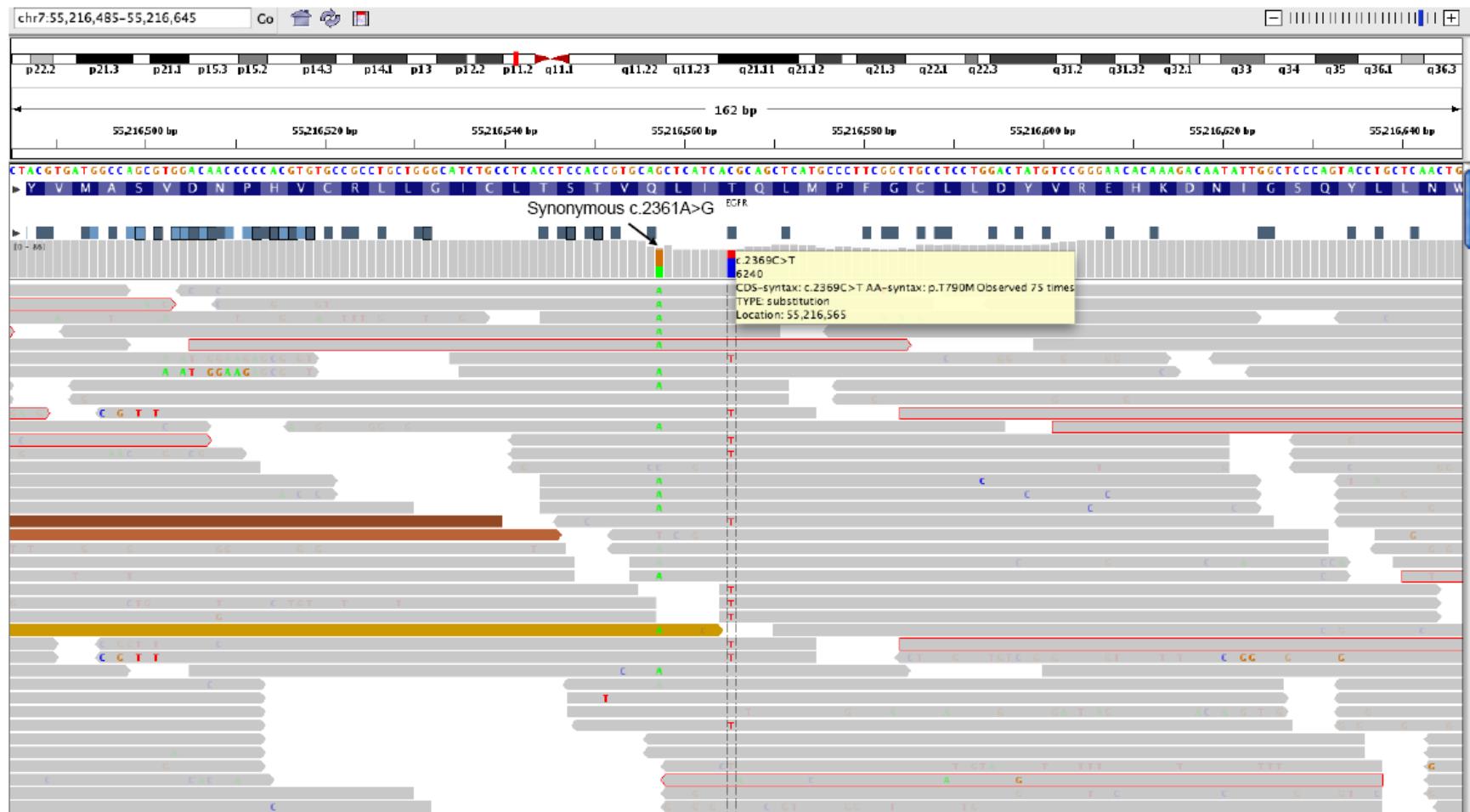


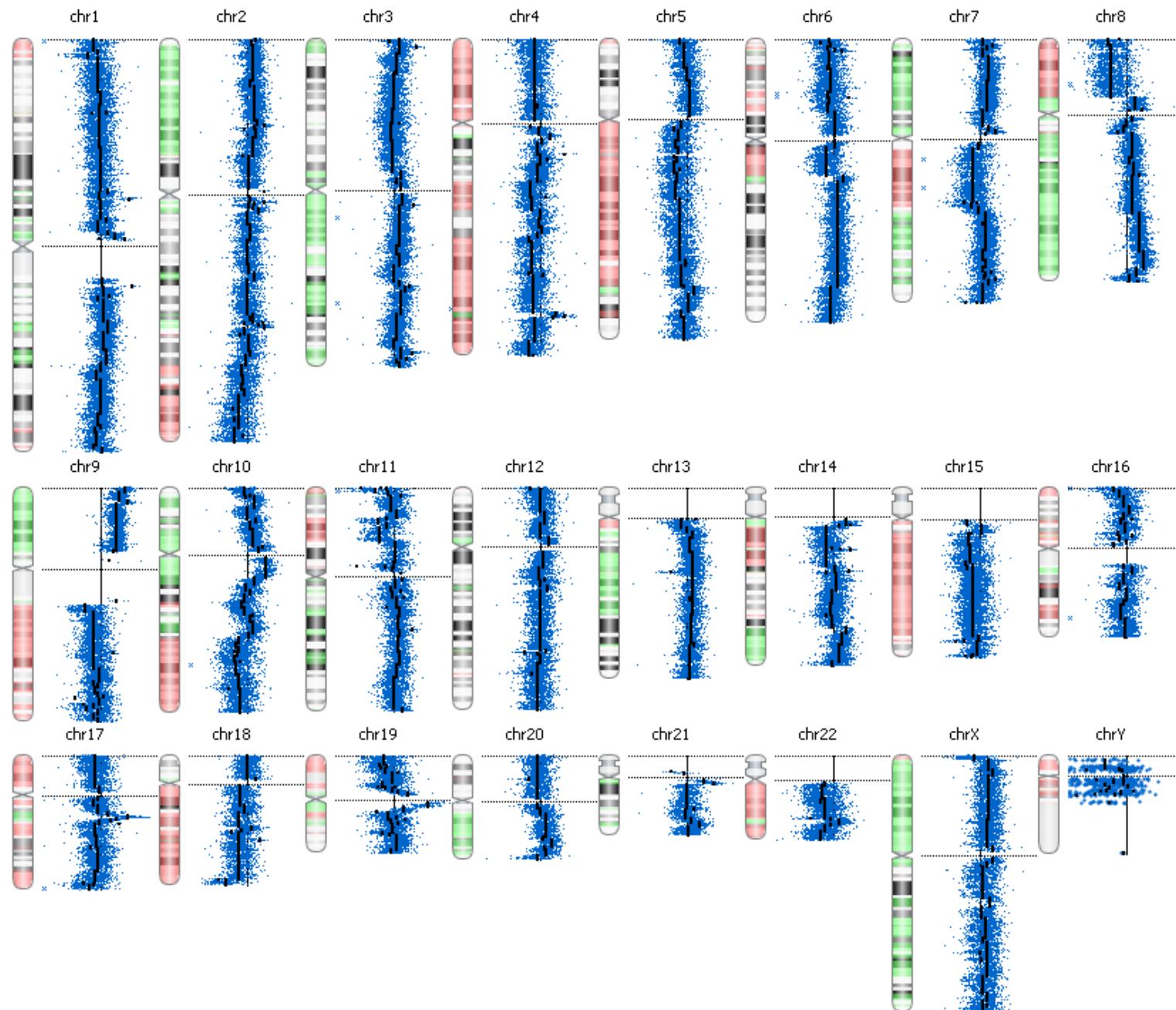
# CMPC11\_2969\_10X



- TUMOR PURITY: ~50%
- DNA YIELD: ~200ng
- RESULT:  
SOLEXA LIBRARY

# CMPC11\_2969 EGFR c.2369C>T p.T790M (Exon 20)





Sample: US82400122\_252206012082\_S01\_CGH\_107\_Sep09\_1\_1

# Patient accrual

First patient enrolled

February 8,2011

Number of pts. screened

106

Number of pts. consented

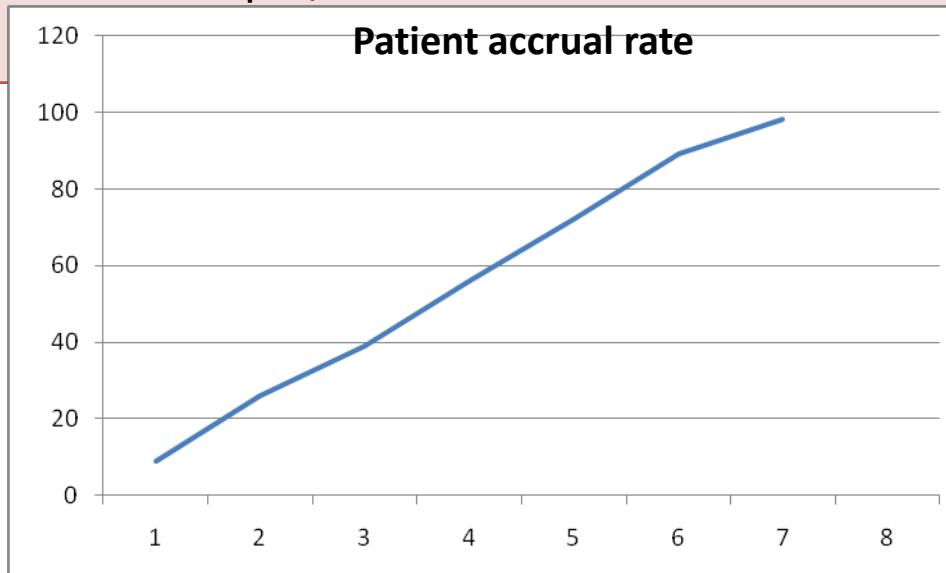
98

Screen failures

2

Average number of pts/week

4



# Baseline patient characteristics

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## Patient characteristics n

Gender : M / F	45/53
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Age: median (range)	59 (24-84)
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Ethnicity:	
Caucasian	70
African-American	13
Asian	12
Hispanic/Latino	3

Smoking Status	
Former	60
Never	34
Unknown	1
Current	3

# Baseline patient characteristics

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## Prior systemic treatments n

One	37
Two	18
> Two Three	25
Previously untreated	18

---

## ECOG performance status

## Smoking status of NSCLC patients

0	13	Former	47
1	73	Current	3
2	11	Never	18
3	1		

# Tumor types

Histology	n
Non-small cell lung cancer	68
Adenocarcinoma	53
Squamous cell	8
Adeno-squamous	2
Large cell	2
NOS	3
Small Cell lung cancer	4
Thymic tumors	26
Thymoma	19
Thymic carcinoma	5
Thymic neuro-endocrine tumor	2

# Tissue collection and complications

## Tissue Collection-Site

Lung	33
Lymph node	17
Soft tissue/Skin	9
Liver	4
Mediastinal mass	5
Pleural fluid	7
Kidney	2
Adrenal	1
Brain	3
Thymus	4
Diaphragm	1
Trachea	1
Bone	2
Endo-bronchial tumor	1
Pending tissue collection	8

## Tissue Collection- Methods

New Biopsies	61
Interventional	44
Radiology	16
Surgery	1
Bronchoscopy	

Archival material	32
Pending	8
Screen failures	3

## Tissue Collection- Complications

Pneumothorax	3 (One Grade 2 and two Grade 1)
Pulmonary hemorrhage	2 (One Grade 2 and one Grade 3)
Vocal cord paralysis	1 (Grade 1)
Dyspnea	1 (Grade 1)
Hypoxia	1 (Grade 2)

## Molecular profile according to tumor type

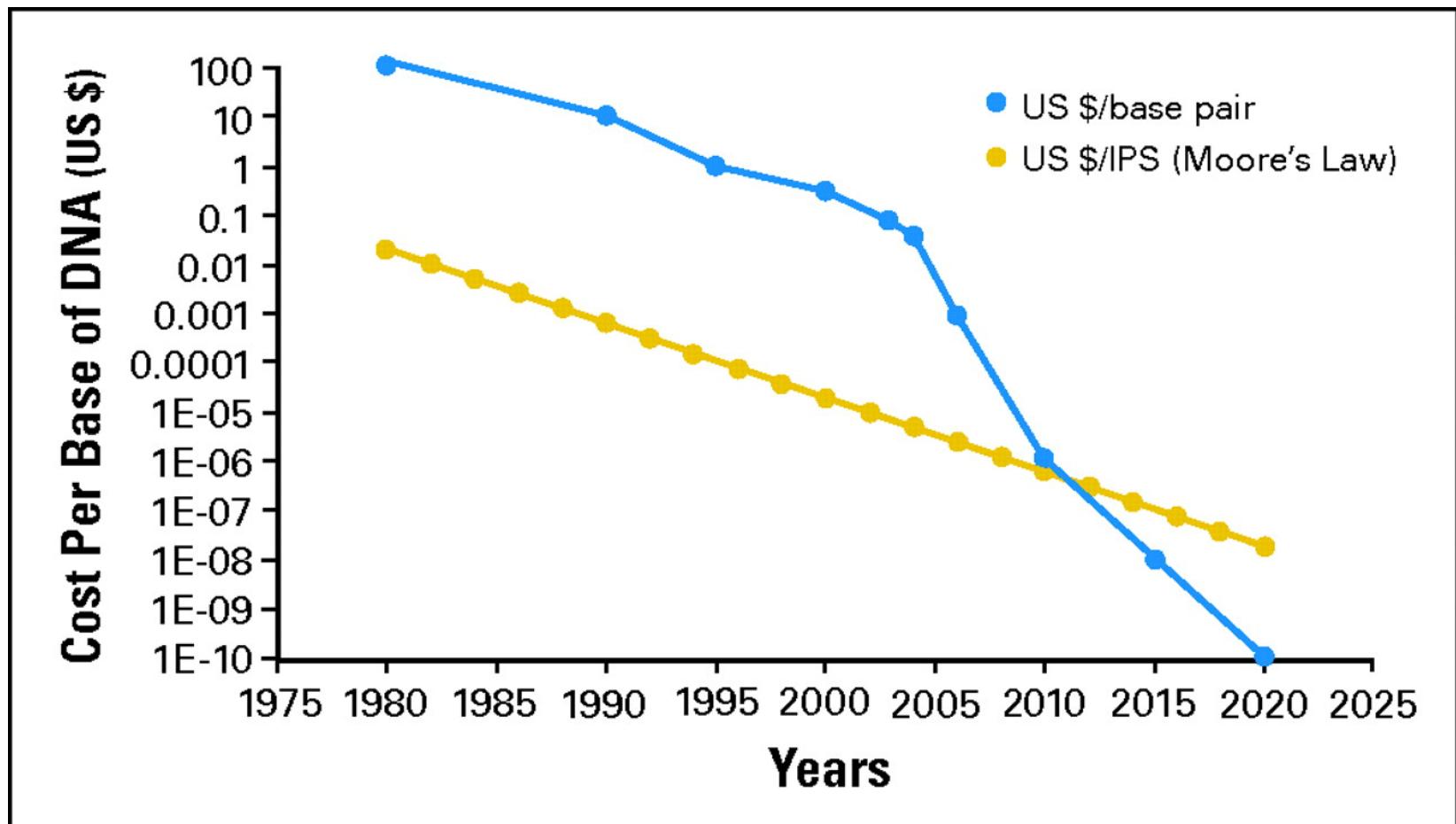
	EGFRm	KRASm	BRAFm	HER2 ampl	EML4- ALK trl	None
NSCLC	12	18	1	3	9	46
SCLC	0	0	0	0	0	4
Thymic	0	0	0	1	0	25

# Treatment arms and responses

		n	PR	SD	PD	NE
Erlotinib	NSCLC	3	2	1	0	0
	SCLC	0				
	Thymic tumors	0				
AZD-6244	NSCLC	6	1	2	2	1
	SCLC	0				
	Thymic tumors	0				
Lapatinib	NSCLC	3	0	0	3	0
	SCLC	0				
	Thymic tumors	0				
MK2206	NSCLC	0				
	SCLC	0				
	Thymic tumors	0				
Sunitinib	NSCLC	0				
	SCLC	0				
	Thymic tumors	0				

NE: Not evaluable

**Advances in massively parallel technologies have dramatically reduced the cost of sequencing.**



MacConaill L E , Garraway L A JCO 2010;28:5219-5228

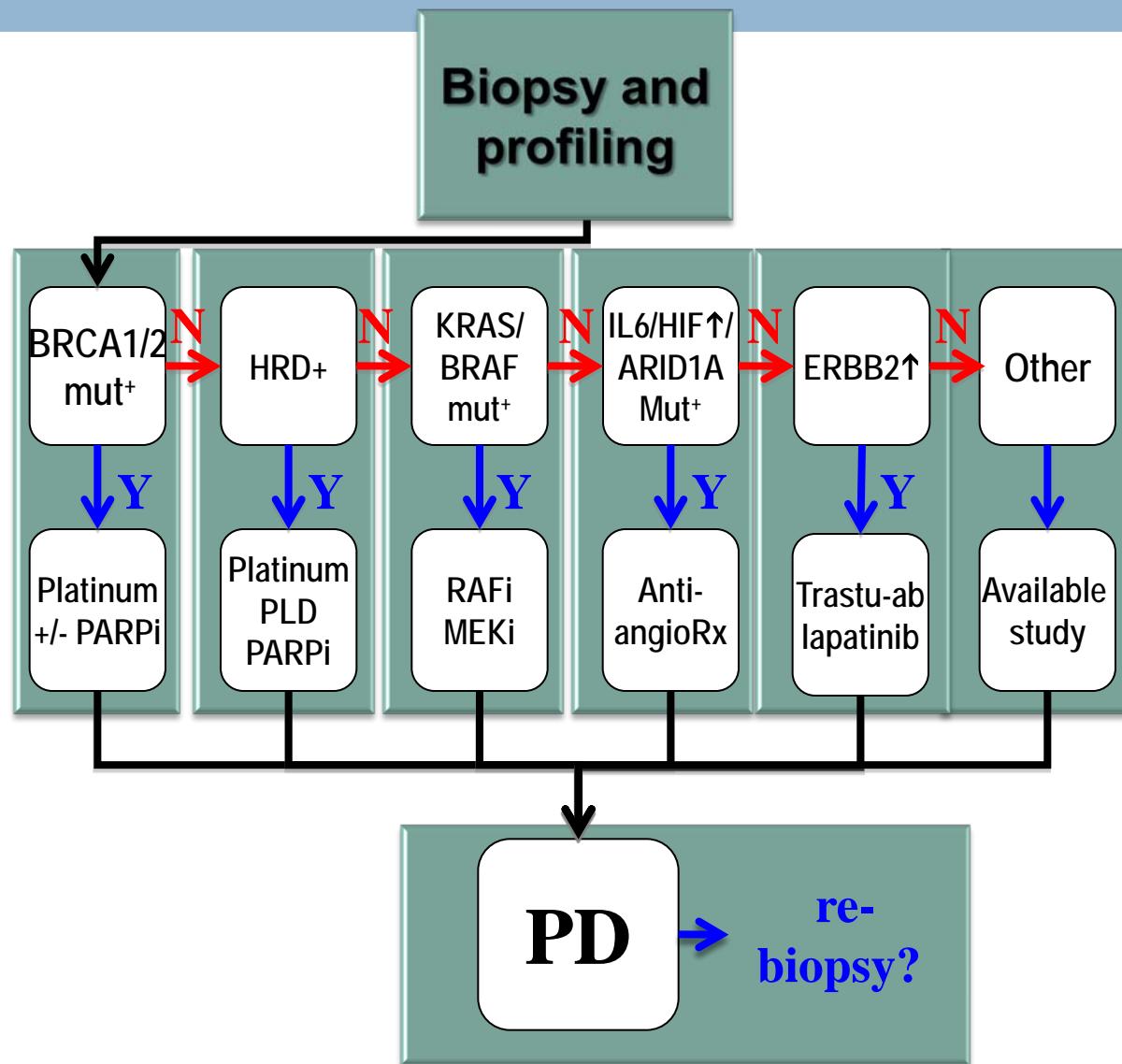
JOURNAL OF CLINICAL ONCOLOGY

# **Progress in Sequencing the Human Genome**

- 2000
  - 12 years
  - 1.800,000,000 USD
- 2010
  - 12 days
  - 20,000 USD
- 2011
  - 5 days
  - 5,000 USD

# Profile-driven targeted therapy selection

## Application to epithelial ovarian cancers







# Acknowledgments

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E. Szabo  
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B. Scepura  
A. Berman  
C. Keen  
M. Manu  
G. Chun

B. Morrow

## Lab

Y. Wang  
J. Voortman  
T. Harada  
H.S. Lee  
I. Petrini  
R. An  
D. Voeller  
T. Pham  
J. Luo

## CCR

P. Meltzer's group  
T. Ried  
M. Raffeld  
S. Pack  
B. Wood  
D. Schrump  
K. Kwong

## CTEP

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H.Chen  
A.Doyle

## University of Pisa

M. Lucchi

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P. Zucali

## VU University Amsterdam

J. Voortman

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